CENTER FOR PATHOPHYSIOLOGY, INFECTIOLOGY AND IMMUNOLOGY
MEDICAL UNIVERSITY OF VIENNA

Center for Pathophysiology, Infectiology and Immunology

Retreat 2021

ALL CONTRIBUTIONS

Thursday, September 30, 2021

Austria Trend Parkhotel Schönbrunn
Hietzinger Hauptstr. 10-14
1130 Vienna

WELCOME NOTE

Dear CePII members!

It is my great pleasure to welcoming you to this year´s retreat of our Center!

Due to the pandemic crisis, two years have passed since we last met and exchanged our scientific knowledge, achievements, and questions. I believe I can say on behalf of all of us: we are more than eager to meet again!

This pandemic affected all of us at different levels. It forced us to live and work in a restricted and isolated way - a so far unexperienced situation, which stands in contrast to all the merits and rules of society, particularly a scientific community.

Nevertheless, I realized that this challenging time did not lead to a standstill or frustrations at our Center; on the contrary, it led to high motivation for excellent research, team spirit, mutual support, and cooperation!

This becomes particularly obvious when looking at the exciting program of this retreat, with excellent abstracts, high-quality publications, and compelling presentations awaiting us. At this point, I would like to thank the scientific board and the administration team for organizing this excellent program!

But since the purpose of a retreat is not only the scientific exchange but also social interactions, our retreat day will end with a great dinner in beautiful ambiance – and for the night owls among us, it may even continue a bit longer ;-).

With this, I am very much looking forward to meeting you all in person, and I want to thank you all for making this day successful and enjoyable!

Kind regards,

Ursula Wiedermann, OELin

PS: 1G + PCR: Please do not forget to bring your vaccination certificate and PCR result!

ORGANIZATION

SCIENTIFIC COMMITTEE

Barbara Bohle
Heimo Breiteneder
Wilfried Ellmeier
Aleksandra Inic-Kanada
Erika Jensen-Jarolim
Alexander Kirschner
Winfried Pickl
Hannes Stockinger
Eva Untersmayr-Elsenhuber
Rudolf Valenta
Ursula Wiedermann-Schmidt

BRIGHT SPARKS JURY

Aleksandra Inic-Kanada
Alexander Kirschner
Winfried Pickl
Eva Untersmayr-Elsenhuber

RETREAT ADMINISTRATIVE ORGANIZATION

Irene Bednar
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LOCATION

Austria Trend Parkhotel Schönbrunn, Hietzinger Hauptstr. 10-14, 1130 Vienna, Austria

PROGRAM

Thursday, September 30, 2021

09.00 – 09.30 **Welcome, opening, and COVID Situation**
Ursula Wiedermann-Schmidt

09.30 – 10:25 **ISPTM – PI Talks**
Chairperson: Ursula Wiedermann-Schmidt
09.30 – 09.45 **Julia Walochnik, David Leitsch und Michael Duchene**
Research in Microbiology & Parasitology at the ISPTM
09.45 – 10.00 **Irma Schabussova und Aleksandra Inic-Kanada**
Mucosal surfaces: Playground for infection and protection
10.00 - 10.15 **Ruth Herbst**
Surface receptor signaling and trafficking in the muscle and immune cells
10.15 – 10.25 **Discussion**

10.25 – 11.20 **IFI – PI Talks**
Chairperson: Wilfried Ellmeier
10.25 – 10.40 **Nicole Boucheron**
Molecular requirements for CD4+ T cell differentiation
10.40 – 10.55 **Katharina Grabmeier-Pfistershammer**
Immune function and deviation - lessons from patients
10.55 – 11:10 **Shinya Sakaguchi**
Transcriptional and epigenetic regulation of effector CD8+ T cell differentiation
11.10-11.20 **Discussion**

11.20 – 12.15 **IPA – PI Talks**
Chairperson: Barbara Bohle
11.20 – 11.35 **Peter Pietschmann**
Osteoimmunology: reciprocal interactions between the immune system and bone
11.35 – 11.50 **Eva Untersmayr-Elsenhuber**
Allergy, inflammation, and cancer – the gut connection
11.50 – 12.05 **Rudolf Valenta**
From the analysis of SARS-CoV-2-specific immune responses towards vaccine development
12.05 – 12.15 **Discussion**

12.15 – 13.00 **Lunch**

13.00 – 13.55 **HAI – PI Talks**
Chairperson: Hannes Stockinger
13.00 – 13.15 **Regina Sommer und Miranda Suchomel**
The Role of Water Hygiene and Medical-technical Hygiene in Infection Prevention
13.15 – 13.30 **Johannes Huppa**
Learning from observation: molecular imaging of T-cell antigen recognition for real change in immunotherapy
13.30 – 13.45 **Anna Repic**
From Rheumatoid arthritis to COVID-19: how our research focus changed in the year of pandemic
13.45 – 13.55 **Discussion**

13:55-14:15 **Coffee Break**

14.15 – 15.39 **Bright sparks talks**

ISPTM and IPA - ***Chairpersons: Aleksandra Inic-Kanada and Eva Untersmayr***

14.15 – 14.22 **Ana Paunkov**
Haemin deprivation renders Bacteroides fragilis hypersensitive to metronidazole

14.22 – 14.29 **Georgii Brazhnikov**
Outer membrane vesicles derived from the probiotic strain E. coli O83 act in TLR4-dependent manner and reduce allergic airway inflammation

14.29 – 14.36 **Nora Geissler**
The role of obesity on allergic reactions and tolerance induction

14.36 – 14.43 **Nicoletta Marquardt**
Generation of anti-hPD-1 and anti-hPD-L1 into caninized mAbs with specific IgG1 and IgG4 canine constant regions by MOE-PCR Ligation-Independent Cloning (LIC)

14.43 – 14.50 **Ines Zettl**
High affine Bet v 1-specific nanobodies interfere with the IgE-Bet v 1 interaction

14.50 – 14.57 **Johanna Rohrhofer**
Anaphylaxis in food allergy depends on a sphingosine-1-phosphate gradient along gastrointestinal epithelial barriers

IFI and HAI - ***Chairpersons: Winfried Pickl and Alexander Kirschner***

14.57 – 15.04 **Lisa Sandner**
The guanine nucleotide exchange factor Rin-like is a novel negative regulator of T follicular helper cell differentiation

15.04 – 15.11 **Peter A. Tauber**
The small molecule inhibitor BX-795 uncouples IL-2 production from inhibition of Th2 inflammation and induces a $CD4^+$ T cell phenotype resembling iTreg

15.11 – 15.18 **Valentina Stolz**
The nuclear receptor corepressor NCOR1 restrains effector $FOXP3^+$ regulatory T cell generation

15.18 – 15.25 **Alexandra Petre**
The Importance of Patient Clusters and Biomarker Populations in the Diagnosis of Interstitial Lung Disease and other Complex Pulmonary Pathologies

15.25 – 15.32 **Michiel Wijnveld**
Novel Human Pathogenic Babesia species Discovered in Austria

15.32 –15.39 **Iris Schachner**
Occurrence and spread of human introduced antimicrobial resistance in a large river water system: Developing a holistic picture for the Danube River

15:39-16:00 **Coffee Break**

16:00-17:00 **Quiz**

17.00 – 17.30 **Retreat Photo Session**

18.00 **CePII Retreat 2021 Dinner** including **Publication Award** ceremony

PI PRESENTERS: BIOSKETCHES

INSTITUT FOR SPECIFIC PROPHYLAXIS AND TROPICAL MEDICINE

Julia Walochnik is associate professor and head of the research unit Molecular Parasitology at the Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria. She studied Biology with a focus on Microbiology and Parasitology and graduated (PhD) at the University of Vienna in 2000. Her research focusses on *Acanthamoeba* spp. and other protozoan pathogens. She has published >200 research articles (Scopus *h*-index: 37) and is the current Editor-in-Chief of *Parasitology Research*. Also, she is on the board of several scientific societies, was the president of the German Society for Protozoology and of the Austrian Society for Tropical Medicine, Parasitology and Migration Medicine in the past and has received several awards, including the Austrian Hygiene Award and the Gerhard-Piekarski Award of the German Society for Parasitology.

David Leitsch studied genetics at the University of Vienna (1996 – 2002) and wrote his master thesis on RNA chaperone regulation of mRNA translation in Escherichia coli under the supervision of Univ. Prof. Udo Bläsi. In October 2003 he started his PhD thesis at the Institute of Specific Prophylaxis and Tropical Medicine on metronidazole toxicity in the anaerobic protist parasite Entamoeba histolytica under the supervision of Prof. Michael Duchêne and received his PhD degree in 2007. David Leitsch has ever since worked on metronidazole toxicity and resistance in anaerobic microbes, having received three grants of the FWF (as of September 2021) and published 20 research papers on this topic alone. From March 2014 – February 2016 David Leitsch was on a research stay as a postdoc at the Institute of Parasitology in Bern, Switzerland, working together with Prof. Norbert Müller. In 2016, he returned to the ISPTM and currently holds a tenure track position (IKV).

Michael Duchêne is Associate Professor at the Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna. He performed his master thesis and PhD thesis (chemistry) at the Max-Planck-Institute of Biochemistry in Martinsried at the Department of Connective Tissue Research finishing in 1984. He then became postdoc at the Gene Center of the University of Munich studying Pseudomonas aeruginosa surface antigens. From 1989 he was employed at the Institute of Specific Prophylaxis and Tropical Medicine. In 1993 he obtained his "venia docendi". His main focus were antigenic structures of Entamoaba histolytica and drug treatment of amoebiasis. In addition, he studied various allergens. During the years he worked with a number of young co-workers including ten PhD students.

Irma Schabussova completed her doctoral research training at the Masaryk University in Brno, CZ and Charles University in Prague, CZ in 2002, focusing on the biology of parasitic worms. Following receipt of a Wellcome Trust Traveling Fellowship, she undertook her post-doctoral fellowship training at the University of Edinburgh, UK where she continued her research training in examining the interaction between parasites and the host immune system. Dr Schabussova joined the Medical University in Vienna, AT in 2006. In 2017, she became an Associate Professor at the Institute of Specific Prophylaxis and Tropical Medicine. In 2019, Dr Schabussova obtained Venia docendi (Habilitation) in the field of Specific Prophylaxis and Tropical Medicine. The research program of Dr Schabussova has focused on dissecting the interaction between parasites and microbiota and the host immune system. Her research also encompasses a significant effort to translate research findings in pre-clinical models into patient-based studies of immune-mediated diseases. Dr Schabussova received funding from FWF and OEAD and she is since many years a dedicated teacher and supervisor of PhD and Master students.

Aleksandra Inic-Kanada graduated in Biochemistry, earned her second MSc in Immunochemistry, and PhD in Immunology, from the University of Belgrade, Yugoslavia. She continued to do science outreach at the Center for Immunological Research established by Prof. Branislav Jankovic and at the Institute of Virology, Vaccines and Sera “Torlak. This was followed by a postdoctoral position at the Medical University of Vienna, where she worked on understanding chlamydial infection pathogenesis and the development of a vaccine against *Chlamydia trachomatis*. From 2014-2018 Aleksandra held the position of deputy scientific director at the Center for Ocular inflammation and infection and earned her habilitation in Immunology and Vaccinology from the Medical University of Vienna. Her research interest focuses on a better understanding the cross-talk between pathogen and epithelial-, structural-, and immune cells (host) required to mediate pathology vs. protection in chlamydial infection.

Ruth Herbst performed her Ph.D. thesis at the Research Institute of Molecular Pathology (IMP) in Vienna and the University of Sheffield, UK. Ruth Herbst joined the laboratory of Steve Burden at the Skirball Institute of Biomolecular Medicine (NYU, New York) as postdoctoral fellow. In 2002 she moved to the Center of Brain Research at the Medical University of Vienna to establish an independent research group. Ruth Herbst and her lab joined the Center of Pathophysiology, Infectiology and Immunology in 2013. The Herbst Lab is interested in how receptors induce intracellular signaling cascades thereby regulating crucial cellular process including cell proliferation, differentiation and survival. In particular, we study the complex molecular intra- and intercellular interactions initiating the formation of the neuromuscular junction. More recently we have also become interested in protein trafficking and the interplay between signaling and protein endocytosis. Our current focus lies on the characterization of GEF-dependent signaling in T cell development and function as well as T cell responses during inflammatory conditions.

INSTITUTE OF IMMUNOLOGY

Nicole Boucheron is junior research group leader at the Medical University of Vienna. Nicole studied Cellular and Molecular Biology in Strasbourg (France) and Milan (Italy). She pursued her PhD at the Ludwig Institute for Cancer Research, Lausanne Branch (Switzerland) and graduated at the University of Lausanne in 2002, followed by postdoctoral studies at the Medical University of Vienna, Institute of Immunology. Nicole has a research interest on the fine-tuning of Th subset functionality in health and disease using mouse models. She has expertise in primary T cell cultures, animal models and flow cytometric methods. In 2014 she received the Sanofi-Aventis prize.

Katharina Grabmeier is a trained clinical immunologist as well as dermatologist with long standing clinical experience in the treatment of patients with (acquired) immunodeficiency but also on immunomodulatory treatment. Within her clinical affiliation she makes part of the interdisciplinary out-patient clinic for adult patients with immunodeficiencies at the Division of Infectious Diseases and Tropical Medicine of the Medical University. Her research interest is the modulation of human immune responses by persistent infections (e.g. by HIV) or therapeutic intervention (e.g. immune check point inhibition, transplantation). A better understanding of how such perturbations affect immune function in individual patients is a prerequisite to improve case management and treatment modalities. The research is strongly influenced by the clinical work as dermatologist and clinical immunologist and is carried out in close collaborations with clinical departments of the Medical University.

Shinya Sakaguchi is Associate Professor at the Institute of Immunology, Medical University of Vienna. He conducted his master thesis (medical science) at the University of Tokyo, Japan, and completed his PhD study (molecular biology) at the University of Vienna in 2009. He performed postdoctoral studies (2009-2016) and later worked as Assistant Professor (2016-2019) at the Institute of Immunology, Medical University of Vienna. He is currently establishing his own independent research group, and his group focuses on transcriptional and epigenetic regulation of cytotoxic T lymphocyte differentiation using mouse models. He received the Karl Landsteiner-Prize awarded from the Austrian Society of Allergology and Immunology in 2010 and the Sanofi-Aventis Prize in 2011. In addition, he was selected for the “Researcher of the month (July 2011)” by the Medical University of Vienna.

INSTITUTE FOR PATHOPHYSIOLOGY AND ALLERGY

Peter Pietschmann was born in Vienna, Austria in 1960. He obtained his MD from the University of Vienna in 1984. He has been trained as a Medical Specialist of Internal Medicine, Rheumatology and Pathophysiology and holds an Associate Professorship ("Habilitation") both for Internal Medicine and Pathophysiology. Since 1999, Peter Pietschmann is the head of a research group which studies the biology and pathophysiology of bone by molecular, cellular and translational approaches in vivo and in vitro. Since 2007, he is the head of the Division of Cellular and Molecular Pathophysiology at the Medical University of Vienna. From 2012 onwards he is the coordinator of the doctoral program "Musculosketetal and Dental Research". Since over 30 years Peter Pietschmann made numerous major and innovative contributions to the field of bone and osteoporosis research. His areas of research include markers of bone turnover, the pathogenesis of secondary osteoporosis, osteoporosis in men, the regulation of bone resorption and the development and characterization of in vivo models for bone research. Based upon his broad experimental and clinical research experience he was among the first to delineate interactions between bone and the immune system, a field now termed "osteoimmunology". Within the area of osteoimmunology, in particular his work relating the pathogenesis of age related osteoporosis to inflammaging and his contributions to bone pathology of inflammatory diseases should be highlighted. Peter Pietschmann has published over 260 papers in peer reviewed journals, 33 book chapters and edited three books. According to Scopus his H-index is 45. He has trained over 50 bachelor, master and doctoral students.

Eva Untersmayr-Elsenhuber studied Medicine at the Medical School of the University of Vienna, Austria and the University of Florence, Italy. In 2001 she started her research carrier focusing on risk factors and mechanisms of food allergy in the research group of Prof. Erika Jensen-Jarolim. Since 2005 she is independent leader of numerous third-party funded research grants focusing on risk factors for food allergic reactions as well as the characterization of novel biomarkers, diagnostic and therapeutic targets in the gastrointestinal tract. Even during her residency in clinical immunology, she established her independent research group on Gastrointestinal Immunology at the Institute of Pathophysiology and Allergy Research in 2007. Eva Untersmayr is author of highly cited publications in peer reviewed journals and winner of several national and international prizes. Currently she is chair of the EAACI Immunology section, member of the EAACI Executive committee and secretary general of the ÖGAI.

Rudolf Valenta graduated at the University of Vienna, Medical Faculty in 1987 in 1987 and received a M.D. degree. From 1993 to 1998, he studied as postdoc at the Department of General and Experimental Pathology, University of Vienna. Since 1993, he has been the Head of the group "Molecular Immunopathology" in the Division of Immunopathology.
Rudolf Valenta has been working in the field of allergy research for more than 30 years. Starting with the molecular and immunological characterization of important allergens, he continued to develop recombinant allergen-based diagnostic tests as well as therapeutic allergy vaccines based on recombinant allergens and genetically engineered hypo-allergens and advanced them into clinical application. He has been awarded several prestigious national and international awards. Rudolf Valenta's work is highly cited (h-index: 99, citations: 36.169 according to Scopus, 21/09/2021), he has published more than 700 original scientific publications, reviews and book chapters, more than 140 patents/patent applications and introduced recombinant allergens into diagnosis and treatment of allergic diseases.

INSTITUTE FOR HYGIENE AND APPLIED IMMUNOLOGY

Regina Sommer is head of the Unit Water Hygiene, where she is teaching, researching and running the accredited Laboratory and Inspection Body "Hygiene Vienna" (authorized by the Ministry of Health). She serves as co-head of the Interuniversity Cooperation Centre (ICC) Water & Health (www.waterandhealth.at). Regina has established with Vetmeduni Vienna the "UV Team Austria" running the Water Test Centre Wiental (www.uv-team-austria.at). Her scientific expertise covers water quality and health, disinfection technologies, water quality in health care facilities and water for medical applications. Besides other 3rd Mission functions Regina is convenor of the Austrian drinking water commission and the board Bathing Water Hygiene (both Ministry of Health) and she represents Austria in the EMEG of the European Commission (EMEG). Since 2019 she is president of the IWA Specialist Group Health Related Water Microbiology. Regina has studied Food- and Biotechnology at the University of Natural Resources and Applied Life Sciences and did her PhD at the Medical University Vienna. In 1999 she received the *Venia docendi* and was appointed as Ao. Univ.-Prof. in 2000.

Miranda Suchomel is head of the Unit Medical-technical Hygiene since 2009 (accredited testing laboratory according to ISO 17025). She has studied Food- and Biotechnology at the University of Natural Resources and Applied Life Sciences, Vienna, and did her PhD at the Medical University Vienna (degree 2009). In 2014 she received the *Venia docendi* and fulfilled the qualification for the degree Associate Professor in 2019. In her research field Miranda is specialized in efficacy testing of hand disinfectants and surface disinfectants. Teaching students and postgraduate training is an important part of Miranda's work, so she runs several courses and gives many lectures. Due to her internationally highly respected scientific expertise she has been appointed as Expert for the World Health Organisation (Task Force on „alcohol-based hand rubs"). Further 3rd

Mission activities comprise participation and guidance of numerous national and international standardisation committees and serving as scientific advisor for professional bodies, as there are the European Interdisciplinary Committee for Hygiene & Compatibility Testing of Medical and the German Verbund für Angewandte Hygiene. Miranda is secretary of the Austrian Society of Hygiene, Microbiology and Preventive Medicine.

Johannes Huppa studied biochemistry in Berlin and moved for his diploma and doctoral thesis to MIT and Harvard Medical School in Boston, where he analyzed the biogenesis and ER-quality control of the TCR-CD3 complex. As a postdoc at Stanford University he devised live-cell and single molecule imaging approaches to study T-cell antigen recognition on a cellular and molecular level. He showed that helper T-cells when in contact with APCs signal through their TCRs over many hours and that such prolonged signaling promotes the integrity of the immunological synapse and the full effector T-cell potential. He quantitated synaptic TCR-antigen interactions via single molecule FRET microscopy. Dr. Huppa started his lab in 2012 in Vienna, where he now combines molecular imaging, synthetic, structural and systems biology to identify and quantitate molecular and cell biological parameters as well as TCR-metrics which determine T-cell responses in health and disease.

Anna Repic holds a Master's degree in Molecular Biology and a PhD in Virology, both from the Comenius University, Bratislava, Slovakia. In December 2009, she joined the group of Prof. Stockinger at the Institute for Hygiene and Applied Immunology (HAI), Center for Pathophysiology, Infectiology and Immunology, as the postdoctoral scientist, and has since successfully contributed to the implementation of the EU project NANOFOL and the approval and realization of the EU follow-up project FOLSMART, which aim to develop novel treatments for rheumatoid arthritis. In 2020, she was awarded an FWF grant in a highly competitive "Urgent funding SARS-CoV-2" call, which has allowed her to establish her own research group. In July 2021, she has been appointed Assistant Professor at HAI. She is the author of 33 peer-reviewed publications and two preprints.

BRIGHT SPARKS TALKS

Haemin deprivation renders Bacteroides fragilis hypersensitive to metronidazole

Ana Paunkov, Katrin Gutenbrunner, József Sóki, and David Leitsch

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Background: The anaerobic bacterial commensal of the human gut *Bacteroides fragilis* can cause severe infections when it reaches other body sites. Haemin is a supplement in the human host and in growth media which allows *B.fargilis* optimal growth. In this study, we tested how haemin impacts sensitivity to metronidazole, an antibiotic frequently used against this bacterium.

Methods: Etest was used to determine the susceptibility of *B.fragilis* with or without haemin supplementation. Oxygen scavenging measurements and aerobic survival assay were employed to determine haemin-specific effects on cells' ability to cope with oxidative stress. Catalase activity and disk diffusion assay were used to evaluate if haemin deprivation leads to higher sensitivity to hydrogen peroxide.

Results: Omission of haemin abolished metronidazole resistance in highly resistant clinical *B*. *fragilis* isolates while resistance to other antibiotics was only mildly or not affected at all. Haemin-deprived cells were more sensitive to oxygen and were unable to remove it from their surroundings. This arguably makes them more susceptible to metronidazole that targets the redox system. Although catalase activity decreased almost 90% in haemin-deprived cells, disk assay did not show direct sensitivity to H_2O_2 possibly because other enzymes involved in H_2O_2 removal still remained active.

Conclusion: Haemin-deprived *B.fragilis* cannot remove oxygen from their surroundings and are highly susceptible to oxygen. These effects make them hypersensitive to metronidazole which is known to cause oxidative damage in the cells. These findings emphasize the importance of monitoring haemin concentration when performing antibiotic resistance studies.

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Outer membrane vesicles derived from the probiotic strain *E. coli* O83 act in TLR4-dependent manner and reduce allergic airway inflammation

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**equally contributed*

Background*:* Probiotic bacteria have been shown to reduce allergic sensitization in mice and humans. However, the fact that they can migrate and replicate in the host has to be considered. The aim of the study was to isolate outer membrane vesicles from the probiotic bacterial strain *E. coli* O83 (EcO83-OMVs) and to study the effect of EcO83-OMVs on the immune system *in vitro* and *in vivo.*

Methods*:* EcO83-OMVs were isolated by ultracentrifugation. HEK293 cells expressing NOD1, NOD2, TLR2, and TLR4 and bone marrow-derived derived dendritic cells (BMDCs) from wild type and TLR4KO BALB/c mice were stimulated with EcO83-OMVs. The production of cytokines was measured by ELISA. Mouse model of ovalbumin-induced airway inflammation was used to study immunotherapeutic effects of EcO83-OMVs.

Results*:* Stimulation of HEK293 NOD1, NOD2, TLR2, TLR4 cells with EcO83-OMVs increased the production of IL-8 suggesting the involvement of these receptors in the signaling by EcO83-OMVs. Stimulation of BMDCs with EcO83-OMVs increased the production of IL-23, IL-12, TNFα, IL-1β, and IL-6, while BMDCs from TLR4KO mice exhibited reduced production of these cytokines. Intranasal application of EcO83-OMVs reduced allergic airway hyperresponsiveness and the level of eosinophils in lungs in comparison to sham-treated controls, but increased the numbers of pulmonary neutrophils, indicating that EcO83-OMVs might cause a shift from Th2 towards Th1 response.

Conclusion: Here we have shown that i) EcO83-OMVs are recognized by NOD1, NOD2, TLR2, and TLR4, ii) production of pro- and anti-inflammatory cytokines is TLR4-dependent, and iii) intranasal application of ECO83-OMVs reduce the development of experimental allergy.

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The role of obesity on allergic reactions and tolerance induction

Nora Geissler[1], Maria Orola[1], Alexander Nardo[2], Thomas M. Stulnig[2], Anna Schmid[1], Maria Nikou[3], Daniela Pollak[3], Elke Korb[1], Irma Schabussova[1], Aleksandra Inic-Kanada[1] and Ursula Wiedermann[1]

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[2] Clinical Division of Endocrinology & Metabolism, Department of Medicine III, Medical University of Vienna, Vienna, Austria
[3] Department of Neurophysiology and Neuropharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

Background*:* Along with allergic diseases, obesity is a severe health problem that is remarkably increasing in prevalence worldwide. Obesity has been recognized as an important risk factor for enhanced allergic reactions. However, the causal link between obesity and allergy is far from fully understood.

Methods*:* C57BL/6 male mice were fed with a high-fat diet (HFD) or standard chow diet (STD), and after nine weeks, mice were immunized and challenged with ovalbumin (OVA). To induce tolerance, mice were orally treated with OVA prior to sensitization. Metabolic parameters and allergen-specific antibodies were measured in serum. The cell differential count was performed in bronchoalveolar lavage (BAL)-cytospins. Cytokine measurements and FACS analysis were performed in lung tissue.

Results*:* HFD-fed animals exhibited increased bodyweight and leptin level in sera compared to STD-fed animals. Sensitization and challenge with OVA resulted in the infiltration of eosinophils and reduced percentage of macrophages in BAL of HFD- and STD-fed mice. Allergen-specific antibody levels were induced in serum in OVA-treated groups. Th2-cytokine levels in the lung supernatants were significantly higher in the allergic group than in the PBS-treated control mice. Furthermore, the HFD-fed group showed a significant increase in the lung, BAL, and serum allergic parameters compared to STD-fed animals. Oral tolerance induction led to a stronger inhibition of the allergic phenotype in obese mice compared to lean ones.

Conclusion: Further evaluation of the impact of HFD on immunological parameters is ongoing. Understanding a cross-talk: obesity – allergy – tolerance may identify novel approaches to combat morbidities associated with Westernized lifestyle.

Supported by: the Austrian Science Fund (FWF) DK-W 1248 – B30

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The guanine nucleotide exchange factor Rin-like is a novel negative regulator of T follicular helper cell differentiation

Lisa Sandner[1], Pol Hoffmann[1], Eleonora Sala[2], Ci Zhu[1], Marlis Alteneder[1], Ramona Rica[1], Birgit Strobl[3], Mirela Kuka[2], Wilfried Ellmeier[1], Ruth Herbst[1], and Nicole Boucheron[1]

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Background: The differentiation of peripheral naïve CD4**+** T cells into specialized T helper subsets is a tightly regulated process to enable an effective T cell mediated adaptive immune response. T follicular helper cells (Tfh) play a critical role in this response, helping B cells produce antibodies against foreign pathogens. Molecular switches called GTPases are controlled by guanine nucleotide exchange factors (GEF) and GTPase-activating proteins (GAP) and were shown to participate in T cell activation. The recently identified factor Rin-like (Rinl) was shown to fulfill GEF function for the Rab subfamily of GTPases which are involved in T cells. Highest expression of Rinl can be found in lymphoid organs making it an interesting target to study in T cell biology.

Methods: We generated Rinl-deficient (Rinl-KO) mice and performed T cell adoptive transfer experiments to study cellular subsets under homeostatic conditions and after immunization or Lymphocytic Choriomeningitis Virus (LCMV) infection by extensive flow cytometry analysis and low input RNA-sequencing.

Results: Our data show that loss of Rinl leads to a T cell intrinsic increase of Tfh upon immunization and infection identifying it as a negative regulator of Tfh cell differentiation. We further identified the involvement of Rinl in the type I interferon pathway, specifically in IFNAR1 cell surface recovery.

Conclusion: Our results unravel a novel important function of Rinl in Tfh differentiation. Given the role of IFNAR1 signaling in Tfh differentiation, it is tempting to speculate that Rinl dampens Tfh differentiation on a molecular level by regulating the IFNAR1 trafficking.

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The small molecule inhibitor BX-795 uncouples IL-2 production from inhibition of Th2 inflammation and induces a CD4$^+$ T cell phenotype resembling iTreg

Peter A. Tauber[1], Bernhard Kratzer[1], Philipp Schatzlmaier[2], Ursula Smole[1], Cordula Köhler[1], Lisa Rausch[3], Jan Kranich[3], Doris Trapin[1], Alina Neunkirchner[1], Maja Zabel[1], Sabrina Jutz[1], Peter Steinberger[1], Gabriele Gadermaier[4], Thomas Brocker[3], Hannes Stockinger[2], Sophia Derdak[5] and Winfried F. Pickl[1,6]

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Background: Interleukin-2 has been shown to be highly important for Treg polarization and function *in vivo*. We screened a panel of small molecule inhibitors including BX-795 that interfere with T lymphocyte signaling pathways for their IL-2 and Treg-inducing potential.
Methods: BX-795 induced changes in secreted cytokine levels and transcription factor expression of T cells upon stimulation were analyzed by cytokine multiplexing and flow cytometry. Effects on gene expression of T cells stimulated in the presence of BX-795 were determined by RNA-seq. Effects of local BX-795 treatment *in vivo*, were studied in mouse models of Th2 inflammation.
Results: BX-795 increased IL-2 mRNA and protein levels in Jurkat T cells and levels of secreted IL-2 by hPBMCs and allergen-specific mouse T cells upon TCR-dependent stimulation. Additionally, BX-795 inhibited the secretion of Th2 cytokines by human PBMCs and murine allergen-specific T cells. RNA-seq analyses revealed close similarity between allergen-induced T cells differentiated in the presence of BX-795 and TGF-□□differentiated iTregs. Although very similar to iTreg, BX-795-induced T cells did not express Foxp3, but overexpressed IL-2, therefore they were designated Th-IL-2 cells. The increased levels of secreted IL-2 were phenotypically relevant, since they caused over-expression of the adenosine-generating 5'-nucleotidase CD73. When applied locally *in vivo*, BX-795 reduced numbers of allergen extract-induced IL-13$^+$T cells, CD4$^+$GATA3$^+$ T cells and eosinophils in two lung models of allergic inflammation.
Conclusions: BX-795 potently increases IL-2 secretion and downregulates type 2 inflammation *in vitro* and *in vivo*. Thus, BX-795 and analogues thereof may be useful for the treatment of type 2 inflammatory disorders such as asthma.

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The nuclear receptor corepressor NCOR1 restrains effector FOXP3$^+$ regulatory T cell generation

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Background: The nuclear receptor co-repressor 1 (NCOR1) is an important regulator bridging chromatin modifying enzymes with transcription factors. We previously showed that NCOR1 controls the survival of developing thymocytes and Th1/Th17 effector function. In the current study we investigated the role of NCOR1 in regulatory T cells (Tregs).

Methods: To study NCOR1 function in Tregs we used conditional KO mice lacking NCOR1 in either all T cells (Cd4-Cre) or specifically in Tregs (Foxp3-YFP-Cre). A variety of immunological *in vitro* methods as well as disease models were performed to analyze the role of NCOR1 in Tregs, including Flow Cytometry, RNA-Sequencing and adoptive CD4$^+$ T cell transfer colitis.

Results: NCOR1-deficient Tregs upregulated effector markers such as ICOS, GITR and KLRG1, which was accompanied with an increased fraction of CD44^{hi}CD62L$^-$ effector Treg (eTreg) subsets. Moreover, NCOR1 deficiency led to increased expression of the transcription factor Myc, an essential driver for eTreg generation. Pharmacological activation of the liver X receptor (LXR), a nuclear receptor whose activity is repressed by NCOR1, lead to increased expression Myc in WT iTregs, suggesting a link between LXRβ and the generation of eTregs. Finally, NCOR1-deficient Tregs failed to protect mice from severe weight loss and intestinal inflammation in adoptive CD4$^+$ T cell transfer colitis.

Conclusion: Our data reveal that a NCOR1/LXR-dependent regulatory pathway controls eTreg differentiation and function.

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Generation of anti-hPD-1 and anti-hPD-L1 into caninized mAbs with specific IgG1 and IgG4 canine constant regions by MOE-PCR Ligation-Independent Cloning (LIC)

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Background: To date, the most successful human anti-cancer immunotherapies include monoclonal antibodies against PD-1 and its ligand PD-L1, while the development of equivalent canine anti-cancer immunotherapies lags far behind. To overcome this drawback, the goal of this project is the generation of caninized anti-hPD-1 and anti-hPD-L1 mAbs with specific canine IgG1 and IgG4 constant regions.

Methods: The identity of human and dog PD-1 and PD-L1 protein sequences was evaluated by pairwise sequence alignment analyses, using the EMBOSS Needle webtool. Furthermore, by use of flow cytometry, we analyzed the binding efficiency of human APC-labelled therapeutic mAbs targeting PD-1 (Pembrolizumab, Nivolumab and Cemiplimab) or PD-L1 (Atezolizumab, Avelumab, and Durvalumab) on the canine macrophage-like cell line DH82, as well as on the human monocytic cell lines THP1 and U937 as controls. By ligation-independent cloning, using the multiple overlap extension PCR (MOE-PCR) strategy, we produced caninized anti-hPD-1 Pembrolizumab and anti-hPD-L1 Atezolizumab mAbs.

Results: Pairwise sequence alignment analyses resulted in: 1) 75.7% identity between human-PD-L1 and canine-PD-L1 (Uniprot Q9NZQ7 vs. E2RKZ5 respectively) and 2) 66.2% identity between human-PD-1 and canine-PD-1 (Uniprot Q15116 vs. A0A024FCJ9, respectively). The FACS analysis resulted in the highest binding efficiency of human APC-labelled anti-hPD-1 Pembrolizumab and anti-hPD-L1 Atezolizumab on the canine macrophage cell line DH82. By use of MOE-PCR, a pVitro1 plasmid containing human variable heavy and light chain sequences and canine constant IgG1 or IgG4 heavy and κ light chain was generated.

Conclusions: Using a ligation independent cloning technique, we show a new strategy to generate caninized anti-hPD-1 and anti-hPD-L1 mAbs, addressing the limitations of available checkpoint inhibitors for the treatment of dog cancers.

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High affine Bet v 1-specific nanobodies interfere with the IgE-Bet v 1 interaction

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Background: Recent studies have shown that administration of allergen-specific monoclonal IgG antibodies significantly reduces allergic symptoms in patients by blocking IgE-binding to the allergen. Since the identification and production of monoclonal blocking antibodies is laborious, we investigated whether allergen-specific nanobodies (Nbs) comprise similar protective properties. Our objective was to generate Nbs specific for Bet v 1, the major birch pollen allergen, and evaluate their potential to inhibit the IgE-Bet v 1 interaction.

Methods: Nbs were isolated from a VHH-cDNA library generated from a camel immunized with Bet v 1 and analyzed regarding their epitope specificity, kinetics, and cross-reactivity to Bet v 1-related allergens (SDS-PAGE, Western Blot, ELISA, surface plasmon resonance). Their efficiency to block specific IgE-binding to Bet v 1 was investigated in ELISA and cell-based assays.

Results: Three Nbs (Nb23, Nb24, Nb32) were isolated that recognize the C-terminal α-helix on Bet v 1 with high affinity (KD = 0.5 - 1.5 x 10-9 M) and cross-react with allergens from alder (Aln g 1) and hazel (Cor a 1). Nbs inhibited the binding of IgE from allergic patients to Bet v 1 and homologues and partially reduced Bet v 1-induced basophil activation.

Conclusion: We isolated high affine Bet v 1-specific Nbs that inhibit polyclonal IgE-binding to Bet v 1 and related allergens indicating protective potential. However, for full blocking of IgE-induced reactions Nbs against other Bet v 1 epitopes will now be generated.

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Anaphylaxis in food allergy depends on a sphingosine-1-phosphate gradient along gastrointestinal epithelial barriers

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Background: Sphingosine-1-phosphate (S1P) was previously reported to facilitate recovery after anaphylaxis by preserving vascular endothelial barrier integrity and by supporting histamine clearance. To evaluate the effect of S1P gradients along gastrointestinal barriers in food allergy, we performed *in vitro*, as well as *in vivo* experiments including a clinical study in food allergic patients.

Methods: In the clinical study plasma was collected from 65 pediatric food allergic patients before diagnostic food challenges. Systemic S1P levels in plasma were measured by mass spectrometry. For *in vitro* assays, barrier integrity of CaCo2 cells was evaluated by transepithelial electrical resistance after apical or basolateral stimulation with S1P. CaCo2 cells were screened for mRNA levels of S1P receptors (S1PRs) by qPCR. Mice were orally sensitized to ovalbumin and intravenously injected with S1P.

Results: Food allergic patients with higher levels of plasma S1P did not experience anaphylaxis during the food challenges. With S1PR2 mRNA being expressed in CaCo2 cells, basolateral S1P stimulation enhanced barrier integrity of the cell monolayer in contrast to apical S1P stimulation. Injecting allergic mice with S1P prior to oral allergen exposure prevented anaphylaxis. Furthermore, reduced levels of the tight junction protein claudin 2 indicated a tighter intestinal epithelial barrier in mice, which have been systemically exposed to S1P.

Conclusion: A S1P gradient along intestinal epithelial barriers regulates barrier integrity. Thus, higher systemic S1P levels strengthen barrier integrity leading to protection against anaphylaxis upon oral allergen exposure.

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The Importance of Patient Clusters and Biomarker Populations in the Diagnosis of Interstitial Lung Disease and other Complex Pulmonary Pathologies

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Background: Interstitial lung disease (ILD) comprises a multitude of clinical entities at times posing significant challenges regarding an accurate diagnosis. The classification systems for ILD are supported by ongoing advances in biomarker and predisposing factor research.

Methods: We developed a data-driven model for patient clustering constituting a supportive tool to increase the diagnostic accuracy. The model is based on surface phenotyping of over 40 markers on immune cells isolated from bronchoalveolar lavage in combination with acquisition of clinical data. Based on the marker expression pattern we constructed an immune cell profile for every participant. We merged the profiles to create a global atlas of the lung immunological microenvironment in various pathologies. The contribution of each participant to the global atlas was assessed with dimensionality reduction tools and the ensuing similarity between profiles was calculated using a nearest neighbouring and partitioning algorithm (PhenoGraph) and/or hierarchical clustering.

Results: Over a period of nine months, we collected 59 consecutive samples from 55 patients undergoing diagnostic bronchoscopy. The initial clinical diagnosis was certain in only 69.5% of cases. Participants presented more than one pulmonary condition in 45.8% of the cases.

Conclusion: Our model enables two distinct approaches. First, assessing the cell population landscape similarity between patients within a diagnostic group allows rapid identification of outlier profiles, which is particularly helpful for cases with uncertain diagnoses. Second, sample clustering is based exclusively on the calculated similarity of the immune cell atlas, thus removing physician bias and introducing the concept of immune atlas nearest neighbours.

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Novel Human Pathogenic *Babesia* species Discovered in Austria

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Background: In Austria three tick species are known to most frequently bite humans: *Dermacentor reticulatus*, *Ixodes ricinus* sensu lato and *Haemaphysalis concinna*. This last species, *H. concinna*, is a poorly studied tick which prompted me to further investigation with the aim to determine the human health risks posed by this species.

Methods: Austrian *H. concinna* ticks were collected either by flagging or from dogs. DNA was extracted and screened using PCR followed by the reverse line blot. Sequencing was employed to confirm results or to further investigate certain signals. Phylogenetic analysis of the babesial 18S rRNA gene and part of the HSP70 gene was conducted using the Geneious Prime software package.

Results: Within *H. concinna*, DNA was detected of several pathogens. Among which were *Borrelia afzelii*, *Babesia* spp., *Rickettsia helvetica* and a *Theileria capreoli*-like organism. Equivocal babesial findings were further investigated. This resulted in the discovery of potentially new *Babesia* species. These new species are closely related to but distinct from *Babesia crassa*, a protozoan originally discovered infecting sheep in Iran.

Conclusions: *Haemaphysalis concinna* is harbouring (DNA of) several pathogens causing Lyme borreliosis, rickettsiosis and babesiosis in humans. Especially the discovery of the unique babesial species was significant. While conducting this study, several human cases were observed in Eurasia with an unknown *Babesia* species with 18S rRNA sequences nearly identical to our findings. Further strengthening the hypothesis that the discovered babesial species is distinct from *Babesia crassa*(-like) and poses a human health risk.

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Occurrence and spread of human introduced antimicrobial resistance in a large river water system: Developing a holistic picture for the Danube River

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Background: The problem of human-induced antimicrobial resistance is an emerging concern for aquatic environments. The isolation of (facultative) pathogenic organisms with acquired antibiotic resistance, even towards last-line antibiotics, from rivers and lakes, is well documented throughout the world but large-scale and quantitative studies are often missing.

Methods: In the course of the last large international survey on the Danube River (Joint Danube Survey 4) a novel quantitative approach on the occurrence and spread of human-induced antimicrobial resistance (resistant bacteria and antibiotic resistance genes) along the whole river and its major propagation factors was applied, integrating a vast number of different environmental and microbiological parameters.

Results: Preliminary results of this comprehensive investigation showed that human faecal pollution is still the most prominent source of microbial pollution in the Danube River, potentially also introducing antibiotic resistant bacteria. The fact that faecal indicator bacteria could be quantified in substantial concentrations in river biofilms along the entire river bank indicates that due to permanent faecal pollution, human derived bacteria can also persist within the river ecosystem. Comparing an initial share of bacterial isolates (n=797) tested for acquired antimicrobial resistances a significant increase in multi-resistance as compared to previous studies was detected.

Conclusion: Based on the findings of this integrative study approach, the current understanding on the importance on the spread and stabilization of human-induced antibiotic resistance in large rivers will be highly improved. The results of this study will also be useful to guide future monitoring and management strategies.

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ALL SUBMITTED ABSTRACTS

Institute of Specific Prophylaxis and Tropical Medicine

1. Characterizing serine phosphorylation of Muscle-specific kinase using a structure-function approach

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Background: Muscle specific kinase (MuSK) is a receptor tyrosine kinase that is crucial for development and maintenance of the neuromuscular junction. It is indirectly activated by motor neuron derived heparansulfate proteoglycan agrin. Its activation initiates acetylcholine receptor clustering, a hallmark of synaptic differentiation. Recently, a novel serine phosphorylation in MuSK was detected that has a modulatory role for MuSK activation. This is exemplified by the fact that phosphomimetic MuSK mutant protein can be activated at lower agrin concentrations. The present study aims to elucidate the pathway behind serine phosphorylation and clarify its exact function.

Methods: We use a crystallography-based approach to study the MuSK protein in active and inactive conformations and to provide insight in phosphoserine modulated MuSK activation.

Results: The intracellular domain of wild type and phosphomimetic MuSK was expressed in Sf-9 insect cells. Both proteins were purified in a multi-step process and characterized with regard to phosphorylation and protein-protein interaction capabilities. Experiments to screen conditions for protein crystallization are currently in progress. Using in silico modelling we observe a conformational change in the activation loop of inactive phosphomimetic MuSK. Thus, we expect a primed conformation in inactive mutant protein, and fully relieved autoinhibition in fully active mutant protein.

Conclusion: Besides resolving the structural basis of MuSK activation, this data will contribute to a comprehensive understanding of MuSK kinase regulation. Understanding the pathway behind serine phosphorylation could open up new prospects to pharmacologically alter postsynaptic differentiation and possibly treat diseases like congenital myasthenic syndrome or myasthenia gravis.

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2. Phylogeographic insights into the first record of the sand fly species *Phlebotomus simici* in Austria

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Background: Phlebotomine sand flies are the principal vectors of *Leishmania* spp. (Kinetoplastida: Trypanosomatidae), the causative agent of leishmaniasis. Data on sand flies in Central Europe is scarce and only one species, *Phlebotomus mascittii*, has been recorded in Austria.
From 2018–2019, entomological surveys were conducted in Austria with the **aim** to further clarify sand fly distribution and species composition.

Methods: Altogether, 66 locations in five federal states of Austria were surveyed with CDC light traps. Caught sand fly specimens were identified by morphological and molecular methods. For phylogeographic analyses, haplotype networks were created and maximum likelihood (ML) analyses were performed.

Results: In 2019, a *Ph. simici* specimen was trapped in Austria for the first time. Analyses of two commonly used marker genes, cytochrome c oxidase I (*coxI*) and cytochrome b (*cytb*), revealed high sequence identity with *Ph. simici* specimens from North Macedonia and Greece. Phylogenetic analyses showed high intraspecific distances within *Ph. simici*, thereby dividing this species into three lineages: one each from Europe, Turkey and Israel. Low interspecific distances between *Ph. simici*, *Ph. brevis* and unidentified *Adlerius* sp. from Turkey and Armenia highlight how challenging molecular identification within the *Adlerius* complex can be.

Conclusion: This study reports the first finding of *Ph. simici* in Austria, representing the northernmost record of this species to date. Moreover, it reveals valuable insights into the phylogenetic relationships among species within the subgenus *Adlerius*. *Phlebotomus simici* is a suspected vector of *L. infantum* and therefore of medical and veterinary importance.

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3. Using micropatterning to establish an in-vitro model of the neuromuscular junction

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The neuromuscular junction (NMJ) is the specialized synapse between motor neuron and skeletal muscle and it is essential for muscle contraction. NMJs are formed as a result of complex interactions between nerve and muscle. During development, motor neurons release a protein called Agrin which is deposited into the basal lamina. Neuronal Agrin activates a signaling cascade in the muscle, leading to the aggregation of the neurotransmitter receptor acetylcholine receptor (AChR). Efficient transmission is ensured by the presence of highly concentrated AChRs at the postsynaptic side and alterations in AChR density and localization lead to pathological conditions, such as myasthenia gravis and congenital myasthenic syndromes. Molecular mechanisms underlying NMJ development are still incompletely understood and shedding light on these processes will help in the development of a successful treatment for neuromuscular diseases.
Studying the NMJ is challenging and cell culture-based approaches are unable to fully recapitulate events that occur in vivo. Current in vitro methods mimic NMJ formation by stimulating cultured myotubes with soluble Agrin, inducing a random distribution of AChR clusters. We want to establish a more physiological assay by immobilizing Agrin on a solid substrate. Using microcontact printing and the SpyCatcher/Tag system, we are able to immobilize recombinant Agrin in a micropattern with defined concentration and localization. Exposure of differentiated myotubes to immobilized Agrin will recapitulate the in vivo condition. With this novel approach, we provide a method that will enable us to better investigate the molecular processes and signalling pathways underlying NMJ formation, maintenance and disease.

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4. Haemin deprivation renders Bacteroides fragilis hypersensitive to metronidazole

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Background: The anaerobic bacterial commensal of the human gut *Bacteroides fragilis* can cause severe infections when it reaches other body sites. Haemin is a supplement in the human host and in growth media which allows *B.fargilis* optimal growth. In this study, we tested how haemin impacts sensitivity to metronidazole, an antibiotic frequently used against this bacterium.

Methods: Etest was used to determine the susceptibility of *B.fragilis* with or without haemin supplementation. Oxygen scavenging measurements and aerobic survival assay were employed to determine haemin-specific effects on cells' ability to cope with oxidative stress. Catalase activity and disk diffusion assay were used to evaluate if haemin deprivation leads to higher sensitivity to hydrogen peroxide.

Results: Omission of haemin abolished metronidazole resistance in highly resistant clinical *B*. *fragilis* isolates while resistance to other antibiotics was only mildly or not affected at all. Haemin-deprived cells were more sensitive to oxygen and were unable to remove it from their surroundings. This arguably makes them more susceptible to metronidazole that targets the redox system. Although catalase activity decreased almost 90% in haemin-deprived cells, disk assay did not show direct sensitivity to H_2O_2 possibly because other enzymes involved in H_2O_2 removal still remained active.

Conclusion: Haemin-deprived *B.fragilis* cannot remove oxygen from their surroundings and are highly susceptible to oxygen. These effects make them hypersensitive to metronidazole which is known to cause oxidative damage in the cells. These findings emphasize the importance of monitoring haemin concentration when performing antibiotic resistance studies.

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5. Prevalence of *Trichomonas vaginalis* co-infection with *Candidatus* Mycoplasma girerdii in Vienna

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Background: *Trichomonas vaginalis*, a flagellate parasitic protozoan is the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection. Moreover, symbiosis with *Mycoplasma hominis* has been reported. *Candidatus* Mycoplasma girerdii, a to date unculturable *Mycoplasma* species, was isolated from vaginal secretions almost exclusively in women infected with *Trichomonas vaginalis*. The aim of the study was to assess the presence/ prevalence of *Ca*. M. girerdii and other *Mycoplasma* species from the vaginal discharge of women in Vienna, as well as from cultured *T. vaginalis*, obtained from patients.

Methods: Vaginal swab specimens were obtained from 319 symptomatic and asymptomatic female patients attending the Outpatients Centre in Vienna. *Mycoplasma* genus-specific 16S rRNA primers were used, followed by sequencing of the amplified gene fragments. 50 *T. vaginalis* isolates collected from female and ten from male patients were included into the study to screen *T. vaginalis* for the presence of *Ca*. M. girerdii.

Results: To date, 67% percent of the sequenced vaginal specimens harbored *Mycoplasma hominis*, 14% were positive for uncharacterized *Mycoplasma spp*. 14% harbored *Ureaplasma parvum* and 1% harbored *U urealyticum*. One *Ca*. M. girerdii sequence was obtained from a patient who was also positive for *T. vaginalis*. None of 20 investigated cultured *T. vaginalis* isolates tested positive for *Ca*. M. girerdii, however 10% harbored *M. hominis*.

Conclusions: High prevalence of genital mycoplasmas implies that alterations of the vaginal microbiota to enhance their survival are feasible. Thereby, the presence of *Candidatus* Mycoplasma girerdii and other unknown species ought to be studied more thoroughly.

6. Effects of acidic and non-acidic cannabinoids on fibroblast-myofibroblast transdifferentiation in McCoy mouse fibroblasts

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Background: In damaged tissue myofibroblasts undergo a phenotype switch described as fibroblast-myofibroblast trans-differentiation (FMT). Deactivating myofibroblasts, exploiting apoptosis in myofibroblasts or reprogramming them into their precursor cells are considered as potential therapeutic approaches to prevent fibrosis. Cannabinoids are considered to have antifibrotic properties. Therefore, we wanted to investigate the effects of non-acidic cannabinoids as well as acidic cannabinoids on TGF-β1 induced FMT.

Methods: McCoy mouse fibroblasts were grown in DMEM. When cells reached 70-80% confluency, a wound healing assay was performed and subsequently cells were treated with cannabinoids (at a concentration of 20 µM or 50 µM) alone with or without LPS (10 ng/mL) or TGF-β1 (10 ng/mL). After 22 hours cells were fixed with 4% PFA. Surface ICAM-1 expression was assessed with modified cell ELISA. Immunofluorescence analysis was conducted via double staining of the cells with fibronectin and cytokeratin 19 antibodies.

Results: Among all cannabinoids, CBDA alone induced wound closure without significant changes in cell morphology at 20 µM and 50 µM. All cannabinoids at 20 µM concentration, except Xe20b, were able to decrease ICAM-1 expression significantly in LPS-stimulated group. Likewise, CBDA and Xe20a at 20 µM concentration caused a significant reduction in ICAM-1 expression in cells stimulated with TGF-β1. THC or THCA alone decreased fibronectin levels at 50 µM concentration. CBD or CBDA alone decreased fibronectin expression and increases cytokeratin-19 expression in stimulated and non-stimulated cells.

Conclusion: Acidic and non-acidic cannabinoids alter the response of McCoy cells when stimulated with TGF-β1 or LPS differently.

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7. Outer membrane vesicles derived from the probiotic strain *E. coli* O83 act in TLR4-dependent manner and reduce allergic airway inflammation

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**equally contributed*

Background*:* Probiotic bacteria have been shown to reduce allergic sensitization in mice and humans. However, the fact that they can migrate and replicate in the host has to be considered. The aim of the study was to isolate outer membrane vesicles from the probiotic bacterial strain *E. coli* O83 (EcO83-OMVs) and to study the effect of EcO83-OMVs on the immune system *in vitro* and *in vivo.*

Methods*:* EcO83-OMVs were isolated by ultracentrifugation. HEK293 cells expressing NOD1, NOD2, TLR2, and TLR4 and bone marrow-derived derived dendritic cells (BMDCs) from wild type and TLR4KO BALB/c mice were stimulated with EcO83-OMVs. The production of cytokines was measured by ELISA. Mouse model of ovalbumin-induced airway inflammation was used to study immunotherapeutic effects of EcO83-OMVs.

Results*:* Stimulation of HEK293 NOD1, NOD2, TLR2, TLR4 cells with EcO83-OMVs increased the production of IL-8 suggesting the involvement of these receptors in the signaling by EcO83-OMVs. Stimulation of BMDCs with EcO83-OMVs increased the production of IL-23, IL-12, TNFα, IL-1β, and IL-6, while BMDCs from TLR4KO mice exhibited reduced production of these cytokines. Intranasal application of EcO83-OMVs reduced allergic airway hyperresponsiveness and the level of eosinophils in lungs in comparison to sham-treated controls, but increased the numbers of pulmonary neutrophils, indicating that EcO83-OMVs might cause a shift from Th2 towards Th1 response.

Conclusion: Here we have shown that i) EcO83-OMVs are recognized by NOD1, NOD2, TLR2, and TLR4, ii) production of pro- and anti-inflammatory cytokines is TLR4-dependent, and iii) intranasal application of ECO83-OMVs reduce the development of experimental allergy.

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8. The role of obesity on allergic reactions and tolerance induction

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Background*:* Along with allergic diseases, obesity is a severe health problem that is remarkably increasing in prevalence worldwide. Obesity has been recognized as an important risk factor for enhanced allergic reactions. However, the causal link between obesity and allergy is far from fully understood.

Methods*:* C57BL/6 male mice were fed with a high-fat diet (HFD) or standard chow diet (STD), and after nine weeks, mice were immunized and challenged with ovalbumin (OVA). To induce tolerance, mice were orally treated with OVA prior to sensitization. Metabolic parameters and allergen-specific antibodies were measured in serum. The cell differential count was performed in bronchoalveolar lavage (BAL)-cytospins. Cytokine measurements and FACS analysis were performed in lung tissue.

Results*:* HFD-fed animals exhibited increased bodyweight and leptin level in sera compared to STD-fed animals. Sensitization and challenge with OVA resulted in the infiltration of eosinophils and reduced percentage of macrophages in BAL of HFD- and STD-fed mice. Allergen-specific antibody levels were induced in serum in OVA-treated groups. Th2-cytokine levels in the lung supernatants were significantly higher in the allergic group than in the PBS-treated control mice. Furthermore, the HFD-fed group showed a significant increase in the lung, BAL, and serum allergic parameters compared to STD-fed animals. Oral tolerance induction led to a stronger inhibition of the allergic phenotype in obese mice compared to lean ones.

Conclusion: Further evaluation of the impact of HFD on immunological parameters is ongoing. Understanding a cross-talk: obesity – allergy – tolerance may identify novel approaches to combat morbidities associated with Westernized lifestyle.

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9. Impact of Senescence of McCOY cells on *Chlamydia trachomatis* infectivity *in vitro*

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Background: The aims of this study were to investigate whether senescent McCoy cells (old passages) are more susceptible to chlamydial infections and to compare their inflammatory response to McCoy cells from younger passages.

Methods: McCoy cells were inoculated with *Chlamydia trachomatis* serovar A (*CtA;* 10^6 IFU ml^{-1}) for 48hrs using two different passages (P6 and P31). Cells were fixed and stained with chlamydia specific FITC labeled LPS antibody. Inclusion bodies (IBs) were enumerated using a fluorescence microscope. Evaluations of Ki67 nuclear positivity, NF-κB activation, DNA damage (γH2AX) using specific antibodies were performed and ICAM-1 expression was measured using a modified cell ELISA.

Results: Old passages infected with CtA exhibited significantly higher IBs than infected younger cells. Cells of old passage showed a higher NF-κB nuclear positivity than younger cells. Furthermore, cells infected with CtA had a stronger NF-κB activation compared to their negative controls. Ki-67 positivity increased in both young and old passages infected with Ct, nevertheless, Ki-67 positivity was lower in old passage compared to young passage. CtA infected cells showed a significant increase of γH2Ax positivity.

Conclusions: Infection of McCoy cells with chlamydiae leads to the increase of senescence markers (ICAM-1, NF-κB, γH2Ax) also in younger cells. These evaluations have to be repeated using disease specific cells like conjunctival or urethral epithelial cells to further assess the possible link of senescence and chlamydial infection. Our data further show that the infectivity of CtA is passage-dependent. This finding suggests to consider the impact of passages in *in vitro* studies.

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10. Galactose metabolism in the parasite Entamoeba histolytica

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The parasite *Entamoeba histolytica* causes amoebic dysentery and liver abscess in humans. The anaerobic protozoon resides in the lumen of the colon where it ferments glucose for energy production. *E. histolytica* possesses an UDP-glucose 4-epimerase (GalE), which catalyses the epimerization of UDP-galactose to UDP-glucose and therefore serves as a bridge between the galactose in the environment and the energy generating glucose metabolism, or alternatively, between glucose and galactose-containing surface antigens of the parasite. Due to these important functions, the recombinant *E. histolytica* GalE was produced in *E. coli*, purified and enzyme activity was measured by an assay based on Michaelis-Menten kinetics and the products were determined directly by reverse-phase HPLC. In addition, we discovered that our *E. histolytica* culture is able to survive in medium containing only galactose instead of glucose. Interestingly, these amoebae differ to the glucose-grown amoebae in shape and in GalE gene expression. We plan to knock down GalE in the parasite to discover more about the impact of this important enzyme, which is considered a drug target in another parasite, *Trypanosoma brucei*. In addition, we began to study other enzymes of the *E. histolytica* sugar metabolism such as the UDP-glucose pyrophosphorylase to potentially discover further drug targets.

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1. **The guanine nucleotide exchange factor Rin-like is a novel negative regulator of T follicular helper cell differentiation**

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Background: The differentiation of peripheral naïve CD4**+** T cells into specialized T helper subsets is a tightly regulated process to enable an effective T cell mediated adaptive immune response. T follicular helper cells (Tfh) play a critical role in this response, helping B cells produce antibodies against foreign pathogens. Molecular switches called GTPases are controlled by guanine nucleotide exchange factors (GEF) and GTPase-activating proteins (GAP) and were shown to participate in T cell activation. The recently identified factor Rin-like (Rinl) was shown to fulfill GEF function for the Rab subfamily of GTPases which are involved in T cells. Highest expression of Rinl can be found in lymphoid organs making it an interesting target to study in T cell biology.

Methods: We generated Rinl-deficient (Rinl-KO) mice and performed T cell adoptive transfer experiments to study cellular subsets under homeostatic conditions and after immunization or Lymphocytic Choriomeningitis Virus (LCMV) infection by extensive flow cytometry analysis and low input RNA-sequencing.

Results: Our data show that loss of Rinl leads to a T cell intrinsic increase of Tfh upon immunization and infection identifying it as a negative regulator of Tfh cell differentiation. We further identified the involvement of Rinl in the type I interferon pathway, specifically in IFNAR1 cell surface recovery.

Conclusion: Our results unravel a novel important function of Rinl in Tfh differentiation. Given the role of IFNAR1 signaling in Tfh differentiation, it is tempting to speculate that Rinl dampens Tfh differentiation on a molecular level by regulating the IFNAR1 trafficking.

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2. Chimeric antigen receptor (CAR) NK cells for the treatment of allergy

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Background: Allergen-specific $CD4^+$ T cells play a central role in the pathogenesis and maintenance of allergic diseases. Currently, patients with allergic diseases are mainly treated with symptomatic therapy, while only few and suboptimal causal therapy options are available. Our aim is to develop a causal treatment of allergies based on the elimination of human allergen-specific $CD4^+$ T cells using CAR cells.

Methods: We created CARs with specificity for allergen-specific $CD4^+$ T cells, consisting of HLA-DR heterodimers chimerized with different intracellular signalling tails (CD3 ζ, CD27, CD28, 4-1BB or Dap10). The different CARs were expressed in Jurkat E6-1 and NK-92 cell lines to investigate their functionality.

Results: The results showed that CARs are well expressed on Jurkat E6-1 cells, with 28–78% of the cells expressing the different CARs when introduced by lentiviral transduction. When the CARs were cross-linked by monoclonal HLA-DR antibodies, the CAR cells increased expression of CD69 up to 15-fold compared to controls. These results allowed us to select the CAR constructs with optimal signalling activity. To further confirm the functionality of the selected HLA-DR CAR, we transduced this construct into the NK-92 cell line. HLA-DR CAR NK-92 cells showed specific lysis of 78% of the target cells comprised by $TRAV17/BV18^+$ Jurkat T cells at 10:1 effecter to target cell ratio.

Conclusion: $HLA\text{-}DR^+$ CAR NK-92 cells show strong cytotoxicity towards allergen specific T cells. Treatment with $HLA\text{-}DR^+$ CAR NK cells may help to reduce the systemic burden of allergen-specific $CD4^+$ T cells in individuals suffering from severe forms of allergies.

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3. The small molecule inhibitor BX-795 uncouples IL-2 production from inhibition of Th2 inflammation and induces a CD4$^+$ T cell phenotype resembling iTreg

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Background: Interleukin-2 has been shown to be highly important for Treg polarization and function *in vivo*. We screened a panel of small molecule inhibitors including BX-795 that interfere with T lymphocyte signaling pathways for their IL-2 and Treg-inducing potential.
Methods: BX-795 induced changes in secreted cytokine levels and transcription factor expression of T cells upon stimulation were analyzed by cytokine multiplexing and flow cytometry. Effects on gene expression of T cells stimulated in the presence of BX-795 were determined by RNA-seq. Effects of local BX-795 treatment *in vivo*, were studied in mouse models of Th2 inflammation.
Results: BX-795 increased IL-2 mRNA and protein levels in Jurkat T cells and levels of secreted IL-2 by hPBMCs and allergen-specific mouse T cells upon TCR-dependent stimulation. Additionally, BX-795 inhibited the secretion of Th2 cytokines by human PBMCs and murine allergen-specific T cells. RNA-seq analyses revealed close similarity between allergen-induced T cells differentiated in the presence of BX-795 and TGF-□□differentiated iTregs. Although very similar to iTreg, BX-795-induced T cells did not express Foxp3, but overexpressed IL-2, therefore they were designated Th-IL-2 cells. The increased levels of secreted IL-2 were phenotypically relevant, since they caused over-expression of the adenosine-generating 5'-nucleotidase CD73. When applied locally *in vivo*, BX-795 reduced numbers of allergen extract-induced IL-13$^+$T cells, CD4$^+$GATA3$^+$ T cells and eosinophils in two lung models of allergic inflammation.
Conclusions: BX-795 potently increases IL-2 secretion and downregulates type 2 inflammation *in vitro* and *in vivo*. Thus, BX-795 and analogues thereof may be useful for the treatment of type 2 inflammatory disorders such as asthma.

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4. The nuclear receptor corepressor NCOR1 restrains effector FOXP3⁺ regulatory T cell generation

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Background: The nuclear receptor co-repressor 1 (NCOR1) is an important regulator bridging chromatin modifying enzymes with transcription factors. We previously showed that NCOR1 controls the survival of developing thymocytes and Th1/Th17 effector function. In the current study we investigated the role of NCOR1 in regulatory T cells (Tregs).

Methods: To study NCOR1 function in Tregs we used conditional KO mice lacking NCOR1 in either all T cells (Cd4-Cre) or specifically in Tregs (Foxp3-YFP-Cre). A variety of immunological *in vitro* methods as well as disease models were performed to analyze the role of NCOR1 in Tregs, including Flow Cytometry, RNA-Sequencing and adoptive $CD4^+$ T cell transfer colitis.

Results: NCOR1-deficient Tregs upregulated effector markers such as ICOS, GITR and KLRG1, which was accompanied with an increased fraction of $CD44^{hi}CD62L^-$ effector Treg (eTreg) subsets. Moreover, NCOR1 deficiency led to increased expression of the transcription factor Myc, an essential driver for eTreg generation. Pharmacological activation of the liver X receptor (LXR), a nuclear receptor whose activity is repressed by NCOR1, lead to increased expression Myc in WT iTregs, suggesting a link between LXRβ and the generation of eTregs. Finally, NCOR1-deficient Tregs failed to protect mice from severe weight loss and intestinal inflammation in adoptive $CD4^+$ T cell transfer colitis.

Conclusion: Our data reveal that a NCOR1/LXR-dependent regulatory pathway controls eTreg differentiation and function.

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5. Improvement of virus-like nanoparticle (VNP)-based hypoallergenic allergy vaccines by membrane-bound cytokines

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Background: A prime candidate for allergy vaccination should not only avoid the release of anaphylactic mediators but also alleviate allergen-specific Th2 dominance. In the context of allergen-specific immunotherapy (AIT), we harnessed a virus-like nanoparticle platform to encapsulate full-length Art v 1, the major mugwort pollen allergen, armed with surface-decorated immunomodulatory cytokines aiming to direct a Th1/Treg response.

Methods: HEK293T cells were co-transfected with expression constructs coding for MAp15 (viral matrix protein) fused to Art v 1, various cytokines fused to the CD16b GPI anchor attachment sequence and MoMLV original gap-pol plasmid for VNP generation. For functional analyses, allergen-specific T cells from Art v 1-specific TCR/DR1 humanized allergy mice were co-incubated with VNP and proliferation was monitored and secreted cytokines were analyzed for signs T cell polarization.

Results: Preliminary results with MA::Art v 1-mIL7::GPI, and MA::Art v 1-mIL-15::GPI decorated VNP co-incubated with allergen-specific T cells showed robust T lymphocyte stimulation compared to the unchaperoned MA::Art v 1 expressing VNPs, and even enhancement in the case of IL-7::GPI, while 'empty' VNPs displaying only the respective cytokines failed to activate Art v 1-specific T cells. Likewise, the ratio of Th1(Treg)/Th2 cytokine production determined through multiplexing was influenced by the cytokine-decorated versions of VNPs containing Art v 1.

Conclusion: Cytokine-decorated VNPs delivering a shielded version of Art v 1 in a hypoallergenic form may prove instrumental in optimizing VNP-based prophylactic and/or therapeutic allergy vaccines.

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6. HDAC1 controls CD8⁺ T cell responses during chronic viral infection

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Background: Chronic viral infections are characterized by the continuous presence of antigens following the primary infection. Due to prolonged antigen stimulation, CD8⁺ T cells progressively loose effector function and acquire an exhausted phenotype. Several subsets of exhausted CD8⁺ T cells are known, however transcriptional and epigenetic mechanisms controlling their differentiation are still poorly understood. Histone deacetylases (HDACs) are key epigenetic regulators and we previously showed that HDAC1 is required for proper CD8⁺ T cell homeostasis and efficient activation and expansion during acute viral infection. However, the role of HDAC1 in chronic viral infection remains elusive.

Methods: We infected mice with a T cell-specific deletion of HDAC1 (*Hdac1*$^{f/f}$ *Cd4*Cre) or mice with HDAC1 deletion in activated CD8⁺ T cells (*Hdac1*$^{f/f}$ *Gzmb*Cre) with the chronic LCMV strain clone 13. Subsequently, flow cytometric analysis of effector CD8⁺ T cell subsets was performed. In addition, we performed bone marrow chimeric approaches combined with infection experiments to study T cell-intrinsic effects.

Results: Our preliminary results show that *Hdac1* deletion in CD8⁺T cells leads to the loss of a CX3CR1⁺ (exhausted effector-like) subset of viral specific CD8⁺ T cells. This corelated with an increased expression of exhaustion markers on viral specific CD8⁺ T cells and increased liver damage. Furthermore, by using a CD8-specific Cre line and by preforming bone marrow chimeric experiment, we show that the loss of CX3CR1⁺ CD8⁺ T cells was due to a CD8⁺ T cell-intrinsic role of HDAC1.

Conclusion: Our data suggest that HDAC1 is a key T cell-intrinsic regulator for exhausted CD8⁺ T cell subset differentiation during chronic viral immune responses.

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7. A robust short-term assay for the determination of cellular reactivity against SARS-CoV-2 after infection and/or vaccination

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Background: The ongoing COVID-19 pandemic has unprecedent detrimental effects on global health and economic systems. To identify immunological non/low-responders, a better understanding and more versatile monitoring of SARS-CoV-2 immunity in convalescent and vaccinated individuals is crucial. While antibody-based tests are widely available, well-evaluated, practical short-term cellular tests are scarce.

Methods: Whole blood (WB) of 97 individuals (40 convalescent patients, 21 vaccinees and 36 healthy controls) was incubated with SARS-CoV-2 spike(S)-, nucleocapsid(NC)- and matrix(M)-protein peptides and evaluated for Th1, Th2, Th17 and inflamatory cytokine secretion and activation-induced marker (AIM) expression. Results were compared to respective cytokine secretion and proliferation assays of gradient isolated PBMCs.

Results: Upon stimulation of WB with NC-peptide mix we identified the combined IL-2 and IL-13 secretion as an ideal biomarker to discriminate between convalescent and control subjects (AUC: 0.944, 95%-CI: 0.8944-0.9945), $p<0.0001$) with a specificity of 90.6 % and a sensitivity of 83.3%. Similar results were obtained upon stimulation with S- or M-peptide mix, however, vaccinees did not respond to S-peptide mix uniformly. Both, severity and time after COVID-19 infection, positively correlated with the recallable cytokine secretion levels. Of the AIMs evaluated, CD69 expression on $CD4^+$ and $CD8^+$ T cells best reflected the SARS-CoV-2 exposure status (AUC: 0.872 and 0.825 (95%-CI: 0.756-0.988 and 0.700-0.950) $p=0.001$ and $p=0.0002$, respectively). Of note, WB cytokine secretion levels significantly correlated with proliferation and cytokine secretion of gradient-isolated PBMCs ($p<0.0001$).

Conclusion: This facile and practical short-term WB assay to determine SARS-CoV-2 T-cell memory response will improve counseling of individuals with incomplete immunity regarding further immunization and/or hygiene measures.

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Institute for Pathophysiology and Allergy Research

1. Investigating the Impact of the Bit Depth of Fluorescence-Stained Images on the Performance of Deep Learning-Based Nuclei Instance Segmentation

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Background: Nuclei instance segmentation can be considered as a key point in the computer-mediated analysis of fluorescence-stained (FS) histological images. Among the diverse computer-assisted approaches, supervised deep learning (DL) methods currently deliver the best performances. Fluorescence images are acquired as greyscale images and the number of grey levels represented in an image is fixed by the bit depth. Many high-end microscopes can acquire images with various image bit depths (mainly 8 bits or 16 bits). Whether the image bit depth affected the performance of DL-based nuclei instance segmentation of FS images was investigated in this study.

Method: We released a fully annotated FS histological image dataset of nuclei at different image magnifications and from five different mouse organs. Using a state-of-the-art DL-based method in combination with different pre-processing techniques, we investigated the impact of image bit depth (i.e., 8 bits vs. 16 bits) on the nuclei instance segmentation performance.

Results: We found very competitive nuclei instance segmentation performances for the models trained with 8-bit and 16-bit images on our new dataset and another publicly available dataset. The new dataset including the raw image patches, as well as the corresponding segmentation masks, is publicly available in the published GitHub repository: https://github.com/masih4/BitDepth_NucSeg

Conclusion: Our results showed very competitive nuclei segmentation performance for the models trained with 8-bit and 16-bit images, which suggested that processing 8-bit images is sufficient for nuclei instance segmentation of FS histological images. This is of advantage when storage place is an issue.

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2. Gene mapping in osteoclasts and osteoblasts: a system biology-based approach to dissect genes encoding RNA-binding proteins

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Bone tissue undergoes constant remodeling throught action of bone forming osteoblasts and bone resorbing osteoclasts, which work together in a highly coordinated manner. Dysregulation of the balanced system results in pathological conditions such as osteoporosis. Central to this study are RNA-binding proteins, which regulate the biology of microRNAs and thus impact on a great variety of cellular processes. In this pioneering study, we will apply a multi-modular systems biology approach to dissect the potential role of a predefined set of genes encoding 180 RNA-binding proteins as cellular players directing the process of bone remodeling. The focus will be given to human primary osteoclasts and osteoblasts under heathy and diseased conditions. Methodologically, the compendium-wide analysis will be done using the GENEVESTIGATOR tool, which consolidates publically available and manually curated transcriptomic data sets. We will analyze information on gene expression patterns, gene-gene associations, regulation of gene expression under perturbations, and dissect osteoblast- and osteoclast-specific gene signatures. Extensive literature search revealed studies, where osteoblasts and osteoclasts were assessed on the transcriptional level; those transcriptomic data sets were incorporated into GENEVESTIGATOR. First results demonstrate that among statistically significant differentially expressed candidate genes *LARP6* and *RBFOX2* are highly expressed in osteoblasts, while for *RBM47* and *ZNF385A* high mRNA expression levels were detected in osteoclasts. Furthermore, hierarchical clustering across all 180 genes revealed a clear separation into four major sub-clusters. We expect that consolidation of data and information gained from each individual analytical module will allow to nominate novel promising candidate genes in osteoblasts and osteoclasts.

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3. A novel model for musculoskeletal aging: *Nothobranchius furzeri,* the turquoise killifish

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Introduction: Despite significant progress in the treatment of age-related osteoporosis, a high number of patients still is undertreated. Therefore, novel animal models for the investigation of the disease are necessary. *Nothobranchius furzeri* is the shortest-lived vertebrate, that can be kept in captivity. Although it is an established model for aging research, studies on bone are lacking. The aim of this study was therefore, to characterize *N. furzeri* as a potential model for musculoskeletal aging.

Material and Methods: Bone properties of aging *N. furzeri* were investigated in male and female fish of the GRZ strain, which were between 8 and 20 weeks old. Bone structure and remodelling were investigated by histology, histomorphometry and micro-computed tomography.

Results: Osteoblasts, mono- and multinucleated osteoclasts but no osteocytes were observed in the vertebral areas. In old males the number of osteoblasts/bone perimeter was decreased (13 ± 6 cells/mm vs. 3 ± 3 cells/mm; $p<0.05$), a similar trend was seen in females. Furthermore, males showed higher bone densities and cortical thickness than females. The weight corrected bone densities of 20 weeks old male *N. furzeri* were significantly lower than in 15 weeks old ones (409 ± 5 mg HA/ccm vs. 643 ± 7 mg HA/ccm; $p<0.001$). In several male and female fish, spinal deformities were observed.

Conclusion: *Nothobranchius furzeri* is a promising model for the study of musculoskeletal aging, in particular with regard to sex-specific differences. The lack of osteocytes is consistent with the fact that *N. furzeri* belongs to the more advanced teleost fish.

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4. Investigation of osteocytic proteins in two different mouse strains by immunohistochemistry

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Background: Bone cells are essential for the regulation of bone homeostasis. Besides osteoblasts and osteoclasts, osteocytes, which are considered as the most abundant cells found inside the bone, are known to have an important part in bone remodelling and homeostasis. In previous publications from our laboratory, differences in the bone microstructure of C3H/J and C57BL/6J mice were described; C3H/J mice exhibited more favourable bone properties. The aim of this study was to determine the protein expression of selected regulators expressed by osteocytes in C57BL/6J and C3H/HeOuJ mice by immunohistochemistry.

Material and Methods: In the present investigation two mouse strains with different bone properties were investigated at the protein level. The expression of the osteocytic proteins sclerostin (SOST), dickkopf-1 (DKK-1) and periostin (POST) were analysed by immunohistochemistry in the femur of 8 and 16 weeks old male C3H/J and C57BL/6J mice. The percentages of osteocytes positive for the respective proteins were assessed by bone histomorphometry.

Results: Differences in the osteocyte protein expression between the mouse strains were seen. C57BL/6J mice showed a significantly higher DKK-1 and SOST expression than C3H/J mice. In contrast, POST expression was significantly higher in C3H/J mice compared with C57BL/6J mice.

Conclusion: DKK-1 and SOST are negative regulators of bone formation, whereas POST promotes osteogenesis. Higher DKK-1 and SOST expression in C57BL/6J mice thus could be responsible for the unfavourable bone properties of this mouse strain. On the other hand, the higher POST expression in C3H/J mice appears to be related with a beneficial effect on bone.

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5. CREB-dependent cAMP signaling in allergic sensitization to birch pollen

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Background: Birch pollen is a significant cause of allergic rhinitis and asthma. Over 60% of birch pollen-allergic patients react exclusively to Bet v 1. Specific lipids from the pollen coat, termed pollen-associated lipid mediators, which are co-delivered along with the allergens, may favor a Th2-dominated immune response. We previously noted that birch pollen total lipids induced phosphorylation of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) in primary human keratinocytes. We hypothesize that airway epithelial cells and antigen presenting cells of atopic and non-atopic subjects show differences in cAMP signaling pathways upon exposure to Bet v 1 and birch pollen lipids.

Methods: Recombinant (r) Bet v 1.0101 was expressed in *E. coli* Nico 21 (DE3) cells, and was purified using standard chromatography techniques. Physiochemical characterization of purified rBet v 1 was performed by circular dichroism (CD) spectroscopy and mass spectrometry. Aqueous pollen extract from birch was produced, and total birch pollen lipids extracted using organic solvents.

Results: The alpha-helical and beta-sheet secondary structure of rBet v 1 were determined by CD spectroscopy. Bet v 1 was observed to be the most abundant protein in the aqueous extract. Total birch pollen lipids loaded on thin layer chromatography plate were visualized using iodine.

Conclusion: The birch pollen total lipids will be characterized using high performance liquid chromatography. The downstream effects of CREB-dependent cAMP signaling, in atopic and non-atopic subjects, in response to exposure of pollen components will be studied using bronchial epithelial cell lines and monocyte-derived dendritic cells.

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6. Food allergen-specific IgG antibodies induced by sublingual treatment with rMald1 and rBetv1

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Background: The majority of birch pollen-allergic individuals develop food allergies because IgE antibodies specific for the major allergen Betv1 cross-react with homologous proteins in various foods, e.g. Mald1 in apple. We have reported that sublingual immunotherapy (SLIT) with recombinant (r) Mald1 induced Mald1-specific blocking IgG which failed to prevent IgEreactivity to Betv1. Furthermore, SLIT with rBetv1 induced antibodies blocking IgE-reactivity to Betv1 but not to Mald1. To better characterize these differing antibody responses, we are in the process to analyze their cross-reactivity and blocking capacity to the Betv1- homologs Cora1 in hazelnut, Pruav1 in cherry and Dauc1 in carrot.

Methods: Allergen-specific IgE, IgG1 and IgG4 levels to rCora1, rPruav1 and rDauc1 were assessed in pre- and post-SLIT samples of 17 rBetv1- and 20 rMald1-treated individuals by ELISA. Basophil activation tests with all allergens were performed with seven birch pollenallergic individuals.

Results: Of all subjects, 95% displayed IgE antibodies to Cora1, 76% to Pruav1 and 27% to Dauc1. Comparing rMald1 with rBetv1-treated individuals, 15/29% developed IgG1 antibodies to Cora1, 30/18% to Pruav1 and 10/12% to Dauc1. Regarding IgG4, 45/53% developed antibodies to Cora1, 40/47% to Pruav1 and 0/23% to Dauc1. So far, we identified six donors for basophil inhibition experiments with Cora1, seven for Pruav1 and one for Dauc1.

Conclusion: SLIT with rMald1 and rBetv1 induced cross-reactive IgG1 and IgG4 antibodies in several individuals. The cross-blocking activity of these food allergen-specific antibodies will be assessed in basophil inhibition experiments.

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7. Generation of anti-hPD-1 and anti-hPD-L1 into caninized mAbs with specific IgG1 and IgG4 canine constant regions by MOE-PCR Ligation-Independent Cloning (LIC)

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Background: To date, the most successful human anti-cancer immunotherapies include monoclonal antibodies against PD-1 and its ligand PD-L1, while the development of equivalent canine anti-cancer immunotherapies lags far behind. To overcome this drawback, the goal of this project is the generation of caninized anti-hPD-1 and anti-hPD-L1 mAbs with specific canine IgG1 and IgG4 constant regions.

Methods: The identity of human and dog PD-1 and PD-L1 protein sequences was evaluated by pairwise sequence alignment analyses, using the EMBOSS Needle webtool. Furthermore, by use of flow cytometry, we analyzed the binding efficiency of human APC-labelled therapeutic mAbs targeting PD-1 (Pembrolizumab, Nivolumab and Cemiplimab) or PD-L1 (Atezolizumab, Avelumab, and Durvalumab) on the canine macrophage-like cell line DH82, as well as on the human monocytic cell lines THP1 and U937 as controls. By ligation-independent cloning, using the multiple overlap extension PCR (MOE-PCR) strategy, we produced caninized anti-hPD-1 Pembrolizumab and anti-hPD-L1 Atezolizumab mAbs.

Results: Pairwise sequence alignment analyses resulted in: 1) 75.7% identity between human-PD-L1 and canine-PD-L1 (Uniprot Q9NZQ7 vs. E2RKZ5 respectively) and 2) 66.2% identity between human-PD-1 and canine-PD-1 (Uniprot Q15116 vs. A0A024FCJ9, respectively). The FACS analysis resulted in the highest binding efficiency of human APC-labelled anti-hPD-1 Pembrolizumab and anti-hPD-L1 Atezolizumab on the canine macrophage cell line DH82. By use of MOE-PCR, a pVitro1 plasmid containing human variable heavy and light chain sequences and canine constant IgG1 or IgG4 heavy and κ light chain was generated.

Conclusions: Using a ligation independent cloning technique, we show a new strategy to generate caninized anti-hPD-1 and anti-hPD-L1 mAbs, addressing the limitations of available checkpoint inhibitors for the treatment of dog cancers.

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8. Effect of roasting on allergenicity of Cor a 9 and Cor a 11 from hazelnut

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Background: Hazelnut allergens (*Corylus avellana*) can induce both, mild allergic symptoms and potentially severe allergic reactions. Recently, the effect of roasting on IgE-binding activity of tree nut allergens was reported. Within this study we evaluated whether roasting of hazelnut allergens (Cor a 9, Cor a 11) affects their allergenicity.

Methods: Raw hazelnuts were subjected to roasting: (15 mins at 180C°). Afterwards raw and roasted hazelnuts were ground, and after , defatting dried at the room temperature. Seed storage proteins were purified from hazelnut extracts by chromatography methods.. The effect of roasting was further evaluated by measurement of specific IgE using 5 sera from hazelnut allergics from a dutch cohort.

Results: We observed several differences between R and DR during the purification process. ELISA confirmed IgE recognition of seed storage proteins (Cor a 9 , Cora 11) in both, R and DR conditions, respectively. However, the effect of roasting showed different effect on Cor a 9 and Cor a 11.

Conclusion: The results suggest different effect of roasting when comparing the igE binding activity of Cor a 9 and Cor a 11. Circular dichroism (CD) spectroscopy and mass-spectrometry analysis will be performed to check physical characteristics. Future investigation of the roasting effect using gastrointestinal cells will be performed.

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9. High affine Bet v 1-specific nanobodies interfere with the IgE-Bet v 1 interaction

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Background: Recent studies have shown that administration of allergen-specific monoclonal IgG antibodies significantly reduces allergic symptoms in patients by blocking IgE-binding to the allergen. Since the identification and production of monoclonal blocking antibodies is laborious, we investigated whether allergen-specific nanobodies (Nbs) comprise similar protective properties. Our objective was to generate Nbs specific for Bet v 1, the major birch pollen allergen, and evaluate their potential to inhibit the IgE-Bet v 1 interaction.

Methods: Nbs were isolated from a VHH-cDNA library generated from a camel immunized with Bet v 1 and analyzed regarding their epitope specificity, kinetics, and cross-reactivity to Bet v 1-related allergens (SDS-PAGE, Western Blot, ELISA, surface plasmon resonance). Their efficiency to block specific IgE-binding to Bet v 1 was investigated in ELISA and cell-based assays.

Results: Three Nbs (Nb23, Nb24, Nb32) were isolated that recognize the C-terminal α-helix on Bet v 1 with high affinity (KD = 0.5 - 1.5 x 10-9 M) and cross-react with allergens from alder (Aln g 1) and hazel (Cor a 1). Nbs inhibited the binding of IgE from allergic patients to Bet v 1 and homologues and partially reduced Bet v 1-induced basophil activation.

Conclusion: We isolated high affine Bet v 1-specific Nbs that inhibit polyclonal IgE-binding to Bet v 1 and related allergens indicating protective potential. However, for full blocking of IgE-induced reactions Nbs against other Bet v 1 epitopes will now be generated.

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10. Generation of recombinant Alt a 1, the major Alternaria alternata allergen for studying ligand transport to immune cells and immunomodulation

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Background: Alternaria alternata sensitization is considered a risk factor for developing asthma. The major allergen Alt a 1, a lipocalin-like protein, is capable to sequester iron-siderophore complexes. We produced recombinant Alt a 1 for transport and activation of aryl hydrocarbon receptor by quercetin-iron complexes in an AZ-AHR human reporter cell line in order to investigate immune regulatory mechanisms of ligand loaded Alt a 1.

Methods: Recombinant Alt a 1 expression was done with Pichia pastoris and pPICZα A plasmid system. Western blotting was performed to characterize the IgE binding to recombinant Alt a 1. Using an AZ-AHR cell reporter assay, hepatoma reporter cells were stimulated with Alt a 1 alone, quercetin alone, or with Alt a 1 loaded with ironquercetin (FeQ2) by measuring luciferase activity.

Results: We produced the pure recombinant Alt a 1 allergen and only the dimer was recognized by serum IgE of Alternaria allergic patients. In the AZ-AHR cell reporter assay, we observed that Alt a 1 alone did not induce luciferase activity. However, the Alt a 1 loaded with iron-quercetin was able to shuttle to the hepatoma AZ-AHR cells leading to increased luciferase activity and activation of the AZ-AHR receptor.

Conclusion: The generated recombinant Alt a 1 was biologically active and able to bind to FeQ2 complex, thereby enabling quercetin-dependent AHR activation with a role in immune tolerance.

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11. Effects of vitamin D analogs in two patient-derived ovarian cancer cell lines

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Ovarian cancer (OC) is one of the most lethal cancers in women. Recent studies suggest that calcitriol may have anticancer activity in OC, but the required pharmacological doses may cause hypercalcemia. In this study, we measured the effect of calcitriol and four synthetic, less calcemic, analogs (PRI-1906, PRI-1907, PRI-5201, PRI-5202) in two OC cell lines (13781 and 14433). CYP24A1 mRNA expression increased in a concentration-dependent manner after treatment with all compounds. After 4h of treatment, 14433 cells were generally more sensitive to all compounds than 13781 cells. In both cell lines, PRI-5202 was the most active compound (in 13781 cells: EC50 = 2.98 ± 1.10 nM, in 14433 cells: EC50 = 0.92 ± 0.20 nM), while PRI-1907 was the least active (in 13781 cells: EC50 = n/d, in 14433 cells: EC50 = 136.2 ± 80,9nM). This difference among the analogs disappeared after 5 days of treatment. Long-term, the 13781 cells became more sensitive to the treatment compared with 14433 cells. All analogs increased CYP24A1 expression more than 10 times in 13781 cells compared with 14433 cells (e.g. PRI-5202: 1.2 ± 0.2 x 10^6 % vs. 0.1 ± 0.05 x 10^6 % of EtOH control). A possible reason for the different sensitivity of the cells could be the 10-times higher basal expression of CYP24A1 in 14433 cells compared with 13781 cells. While VDR staining intensity was significantly upregulated after treatment with almost all analogs in 13781 cells ($p < 0.05$ vs. EtOH control), only PRI-5201 ($p < 0.05$ vs. EtOH control) increased VDR expression significantly in 14433 cells. Importantly, after 5 days of treatment, all compounds significantly reduced cell number in the 13781 cell line, while in the 14433 cell line only PRI-1907, PRI-5201 and PRI-5202 had significant effect. The most active was again analogue PRI-5202.

Conclusion: All tested analogs work in OC cells, but the effect is cell line- and analog-dependent. PRI-5202 was the most active in increasing CYP24A1 expression and reducing cell number. These data are promising because this analog has already been proven less calcemic than calcitriol in mice. Further studies are needed to test its anticancer potential against OC in vitro and in vivo.

12. Rounding off the farm effect: beta-lactoglobulin with zinc counterbalances a pro-allergic Th2-response via lipocalininteracting-membrane-receptor (LIMR) on innate immune cells

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Background: Our previous studies demonstrated that the in vitro and in vivo immune response mediated by the whey protein beta-lactoglobulin (BLG) depends on its loading status. We have identified zinc as a major binding partner of BLG, which we found in stable dust and ambient air of cattle farms. Here, we investigated the effect of BLG-zinc on the immune response, and the potential cellular uptake mechanism and receptor of BLG.

Methods: BLG-zinc or BLG depleted of zinc was incubated on healthy donor PBMC, and cell proliferation and cytokine patterns were measured via flow cytometry. The expression of the lipocalin-interacting-membrane-receptor (LIMR), a potential BLG receptor, was evaluated on PBMC subsets. Uptake of FITC-labelled BLG was investigated simultaneously to LIMR expression in monocytic THP-1 cells.

Results: Stimulation of PBMC with BLG-zinc resulted in reduced proliferation, lower IL-4 and IL-5, and higher IFN-γ release compared to stimulation with BLG only. LIMR was expressed on CD14**+** monocytes and CD56**+** NK cells. Stimulation of THP-1 cells resulted in a concentration-dependent decrease of LIMR with increasing BLG-FITC concentration.

Conclusion: BLG-zinc reduced PBMC proliferation and counter-balanced the cytokine milieu towards Th1. The putative BLG-receptor LIMR was primarily expressed by innate monocytic and NK cells, the latter known to release the major Th1-cytokine IFN-γ. BLG uptake negatively correlated with LIMR membrane expression in THP-1 cells. Our study, therefore, indicates a significant antigen independent contribution of BLG-zinc to the allergy-protective farm effect.

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13. Anaphylaxis in food allergy depends on a sphingosine-1-phosphate gradient along gastrointestinal epithelial barriers

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Background: Sphingosine-1-phosphate (S1P) was previously reported to facilitate recovery after anaphylaxis by preserving vascular endothelial barrier integrity and by supporting histamine clearance. To evaluate the effect of S1P gradients along gastrointestinal barriers in food allergy, we performed *in vitro*, as well as *in vivo* experiments including a clinical study in food allergic patients.

Methods: In the clinical study plasma was collected from 65 pediatric food allergic patients before diagnostic food challenges. Systemic S1P levels in plasma were measured by mass spectrometry. For *in vitro* assays, barrier integrity of CaCo2 cells was evaluated by transepithelial electrical resistance after apical or basolateral stimulation with S1P. CaCo2 cells were screened for mRNA levels of S1P receptors (S1PRs) by qPCR. Mice were orally sensitized to ovalbumin and intravenously injected with S1P.

Results: Food allergic patients with higher levels of plasma S1P did not experience anaphylaxis during the food challenges. With S1PR2 mRNA being expressed in CaCo2 cells, basolateral S1P stimulation enhanced barrier integrity of the cell monolayer in contrast to apical S1P stimulation. Injecting allergic mice with S1P prior to oral allergen exposure prevented anaphylaxis. Furthermore, reduced levels of the tight junction protein claudin 2 indicated a tighter intestinal epithelial barrier in mice, which have been systemically exposed to S1P.

Conclusion: A S1P gradient along intestinal epithelial barriers regulates barrier integrity. Thus, higher systemic S1P levels strengthen barrier integrity leading to protection against anaphylaxis upon oral allergen exposure.

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14. Stereospecific Activation of the Calcium-Sensing Receptor (CaSR) Induces Inflammation in Colon Cancer Cells

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Background: Recent studies suggested that activation of the Calcium-Sensing Receptor (CaSR) induces inflammation in the colon. The aim of this study was to prove that this pro-inflammatory effect is indeed mediated through the CaSR, by using stereospecific pharmacological modulators of the CaSR in colon cancer cell lines.

Methods: We used HT29 colon cancer cells stably transduced with either the CaSR fused to GFP or GFP only. Cells were treated with the active (R) or inactive (S) enantiomers of either the CaSR-activating calcimimetic NPS 568, the CaSR-inhibiting calcilytic NPS 2143, or a combination of both.

Results: Stimulation of the HT29-CaSR-GFP cells with NPS R-568 increased IL-8 gene (RT-qPCR) and protein (ELISA) expression, while the CaSR-unselective NPS S-568 did not. Pre-treatment of the cells with the CaSR-selective antagonist NPS R-2143 inhibited the pro-inflammatory effect of NPS R-568 (p<0.001), which was not observed with the CaSR-unspecific NPS S-2143. The combination of S-568 and S-2143 did not have any effect. None of the treatments showed any effect in HT29-GFP cells, lacking the CaSR.

Conclusions: We have proven that the pro-inflammatory effect of a calcimimetic in colorectal cancer cells is indeed mediated through the CaSR, rather than receptor-unspecific effects of the drug. These findings implicate the CaSR as driver of intestinal inflammation and thus potentially malignant changes. This is also the first time that stereospecific modulation has been used to explore the role of the CaSR in specific cell types and provides an important tool for future investigations.

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15. Anticancer activities of *Curcuma longa* and the possible mechanism in prostate cancer cells

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Background: Prostate cancer (PC) is currently the second most common cause of cancer-related death among men worldwide. Prevalent treatment of PC including hormone and radiotherapy, are often accompanied by side effects. Therefore, new therapies that demonstrate efficacy in PC treatment with less associated side effects are still in urgent need. *Curcuma longa* is a medicinal herb reported to have anticancer activity and low cytotoxicity.

Methods: The herb powder was supported by local pharmacy. The main compounds were identified using UHPLC-QTOF-MS/MS chromatography. We used the human PC cell lines DU145 and PC3, and the prostate epithelial cell line PNT2 to perform cell viability assays. Quantitative real-time PCR were performed to demonstrate the expression levels of multiple cancer-related genes. Illumina-sequencing gene expression analysis was used to display specific gene expression patterns and to understand the mechanism of anticancer activities of *C. longa*.

Results: After 48h treatment of *C. longa*, the viability of both DU145 and PC3 cells were inhibited dose-dependently. In RT-qPCR experiments, significant upregulation of p21, TMEM79 and ACOXL in the treatment groups can be observed, comparing to the non-treated groups. TMEM79 and ACOXL were found to be much highly enriched in PNT2 cells than in DU145 cells.

Conclusion: *C. longa* suppressed the proliferation of DU145 and PC3 cells, reduced their cell viability in comparison with PNT2 cells, which suggests that it may be effective for treating PC. TMEM79 and ACOXL were expressed significantly higher in PNT2 cells than in DU145 cells and could be novel biomarker candidates for PC diagnosis.

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1. Mechanical Forces involved in T-cell antigen recognition

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Background: Efficient scanning of tissue that T-cells encounter during their migratory life is pivotal to protective adaptive immunity. In fact, T-cells can detect the presence of even a single antigenic peptide/MHC complex (pMHC) among thousands of structurally similar yet non-stimulatory endogenous pMHCs on the surface of antigen-presenting cells (APCs) or target cells. Mechanical forces acting on ligand-engaged T-cell receptors (TCRs) have previously been implicated in T-cell antigen recognition, yet their magnitude, spread, and temporal behaviour are still poorly defined.

Methods: We employed single-molecule sensitive microscopy setups monitoring the optical parameters of molecular force sensors attached to the base of the ligand of interest and anchored to a glass-supported lipid bilayer. The FRET-based sensor was either equipped with a TCR reactive single chain antibody fragment or a peptide-loaded MHC, the physiological TCR ligand. Observed force kinetics and amplitudes were analysed for their role in T-cell antigen detection and effector functions.

Results: When confronting T-cells with gel-phase SLBs we observed both prior and upon T-cell activation a single, well-resolvable force-peak of approximately 5 pN and force loading rates on the TCR of 1.5 pN per second. When facing fluid-phase SLBs instead, T-cells still exerted tensile forces yet of threefold reduced magnitude and only prior to but not upon activation.

Conclusions: The study is the first of its kind in which forces exerted on single TCRs have been quantitated and tracked within the immunological synapse, and the results challenge current paradigms regarding cell-cell recognition events in immunology.

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2. The Importance of Patient Clusters and Biomarker Populations in the Diagnosis of Interstitial Lung Disease and other Complex Pulmonary Pathologies

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Background: Interstitial lung disease (ILD) comprises a multitude of clinical entities at times posing significant challenges regarding an accurate diagnosis. The classification systems for ILD are supported by ongoing advances in biomarker and predisposing factor research.

Methods: We developed a data-driven model for patient clustering constituting a supportive tool to increase the diagnostic accuracy. The model is based on surface phenotyping of over 40 markers on immune cells isolated from bronchoalveolar lavage in combination with acquisition of clinical data. Based on the marker expression pattern we constructed an immune cell profile for every participant. We merged the profiles to create a global atlas of the lung immunological microenvironment in various pathologies. The contribution of each participant to the global atlas was assessed with dimensionality reduction tools and the ensuing similarity between profiles was calculated using a nearest neighbouring and partitioning algorithm (PhenoGraph) and/or hierarchical clustering.

Results: Over a period of nine months, we collected 59 consecutive samples from 55 patients undergoing diagnostic bronchoscopy. The initial clinical diagnosis was certain in only 69.5% of cases. Participants presented more than one pulmonary condition in 45.8% of the cases.

Conclusion: Our model enables two distinct approaches. First, assessing the cell population landscape similarity between patients within a diagnostic group allows rapid identification of outlier profiles, which is particularly helpful for cases with uncertain diagnoses. Second, sample clustering is based exclusively on the calculated similarity of the immune cell atlas, thus removing physician bias and introducing the concept of immune atlas nearest neighbours.

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3. Automated flow cytometry for near real-time monitoring of bacteria in drinking water resources

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Legally required methods for assessing microbiological drinking water quality, e.g. faecal indicator bacteria, are time-consuming and cannot be used for operational real-time monitoring. In practice, chemophysical surrogate parameters are determined, providing only indirect information. To directly characterize microbiological properties of drinking water, the determination of total bacterial cell counts (TCC) via flow cytometry is increasingly being used.

The main aim of this project is to enable for the first time a high resolution in-sight into the temporal dynamics of microbiological properties of two main drinking water resource types, karstic spring water and porous groundwater. This will be achieved by evaluating the measurement accuracy, sensitivity and reliability of a novel fully automated flow cytometer (BactoSense) for the parameter TCC, as well as high and low nucleic acid content bacteria.

With project progress, new functions will be added, allowing for the distinction of intact and active cells. The device will be tested in the laboratory and natural habitats: riverine groundwater bodies, karstic springs, water pipes, laboratory microcosms, being challenged with natural fluctuations of water properties and artificial pollution scenarios. Based on the collected data, an event triggered automated sampling (ETAS) algorithm will be developed, constituting a direct link between monitoring activities of drinking water suppliers and legally required surveillance parameters by automatically sampling water for traditional microbiological analysis in case of pollution.

Combining the obtained data from field surveys with environmental parameters will enable a deeper understanding of microbial properties and disturbances of drinking water resources, relevant for drinking water safety.

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4. Novel Human Pathogenic *Babesia* species Discovered in Austria

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Background: In Austria three tick species are known to most frequently bite humans: *Dermacentor reticulatus*, *Ixodes ricinus* sensu lato and *Haemaphysalis concinna*. This last species, *H. concinna*, is a poorly studied tick which prompted me to further investigation with the aim to determine the human health risks posed by this species.

Methods: Austrian *H. concinna* ticks were collected either by flagging or from dogs. DNA was extracted and screened using PCR followed by the reverse line blot. Sequencing was employed to confirm results or to further investigate certain signals. Phylogenetic analysis of the babesial 18S rRNA gene and part of the HSP70 gene was conducted using the Geneious Prime software package.

Results: Within *H. concinna*, DNA was detected of several pathogens. Among which were *Borrelia afzelii*, *Babesia* spp., *Rickettsia helvetica* and a *Theileria capreoli*-like organism. Equivocal babesial findings were further investigated. This resulted in the discovery of potentially new *Babesia* species. These new species are closely related to but distinct from *Babesia crassa*, a protozoan originally discovered infecting sheep in Iran.

Conclusions: *Haemaphysalis concinna* is harbouring (DNA of) several pathogens causing Lyme borreliosis, rickettsiosis and babesiosis in humans. Especially the discovery of the unique babesial species was significant. While conducting this study, several human cases were observed in Eurasia with an unknown *Babesia* species with 18S rRNA sequences nearly identical to our findings. Further strengthening the hypothesis that the discovered babesial species is distinct from *Babesia crassa*(-like) and poses a human health risk.

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5. A Novel Flow Cytometric Approach for the Quantification and Quality Control of Chlamydia trachomatis Preparations

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Chlamydia trachomatis (Ct) is an obligate intracellular bacterium with a biphasic developmental cycle manifesting two morphological forms: infectious elementary bodies (EBs) and replicative intracellular reticulate bodies (RBs). Density gradient centrifugation of infected cell lysates is the state-of-the-art method to isolate both. Current protocols for quantification of the isolates assess infectious particles by titering inclusion-forming units, using permissive cell lines, followed by immunofluorescence analysis. Enumeration of total particle counts is achieved by counting labeled EBs/RBs using a fluorescence microscope. Both methods are time-consuming with a high risk of observer bias. For a better assessment, we developed a simple and time-saving flow cytometry-based method to quantify *Ct* preparations. We established an approachable workflow and implemented optimized flow cytometry settings for measuring small particles, such as EBs with a size of 300 nm. This included optimization of gain and threshold settings with addition of a neutral density filter for small-particle discrimination. The nucleic acid dye SYBR® Green I (SGI) was used together with propidium iodide and 5(6)-carboxyfluorescein diacetate to enumerate and discriminate between live and dead bacteria. We found no significant differences between the direct particle count of SGI-stained *Ct* preparations measured by microscopy or flow cytometry ($P > 0.05$). Furthermore, we completed our results by introducing a cell culture-independent viability assay. Our measurements showed excellent reproducibility and comparability to the existing state-of-the-art methods, indicating that the evaluation of *Ct* preparations by flow cytometry is a fast and reliable method. Thus, our method facilitates an improved assessment and quality control of *Ct* preparations.

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6. Revealing the molecular basis underlying T cell antigen recognition in health and autoimmunity

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Thymic selection is essential for shaping the T-cell antigen receptor (TCR) repertoire. To ensure central tolerance developing T cells undergo with their newly recombined TCRs negative selection, in which T cells with high affinity TCRs towards self-antigens become eradicated. However, as recently demonstrated clonal deletion does not efficiently remove all self-specific T cells, which can cause harm if not under control by peripheral tolerance mechanisms. We hypothesize that autoreactive T cells, i.e. self-specific T-cells which have breached central tolerance, differ significantly from non-self-specifc T cells with regard to TCR:antigen binding, in particular because of alterations within the immunological synapse, the transient interface between the T-cell and its antigen presenting cell (APC). To test this, we compared the synaptic TCR-pMHC binding dynamics as they occur for auto-reactive T cells, to pathogen-specific T cells with the use of a Förster Resonance Energy Transfer (FRET)-based molecular imaging system. To this end, we generated TCR-transgenic T-cells with an auto-reactive preproinsulin-specific TCR (1E6) as well as an public, CMV-specific TCR (RA14) by CRISPR-Cas9 mediated TCR replacement. Correlating TCR:pMHC binding *in situ* and *in vitro* with the ensuing T-cell response will be instrumental to reveal the molecular basis underlying antigen recognition in health and autoimmunity.

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7. From TCR to CAR and back, TCR-based CARs outperform conventional CARs in Sensitivity

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CAR-T cell therapies have shown to be potent tools in the fight against cancer. However, a complication associated with current CAR designs is limited antigensensitivity. CAR-T cell sensitivity toward antigen is approximately 1,000-times reduced as compared to TCR-mediated recognition of nominal peptide–HLA complex. Consequently, antigen escape variants can emerge which results in cancer relapse. To overcome this impediment, we are devising CARs with the TCR/CD3 complex as framework. For proof of concept and system optimization we derive the ligand recognition domain from the T1-scFv which targets HLA-A201/NY-ESO-1 as model antigen and which can be precisely fine-tuned using NY-ESO-1-derived altered peptide ligands within an affinity spectrum covering 4 orders of magnitude. We customize a planar glass-supported lipid bilayer (SLB)-based system which can be decorated with proteins of choice with densities of choice to serve as surrogate target cell. We show that several TCR-based CAR designs outperform conventional CARs in sensitivity when targeting high affinity ligands. Additionally, TCR-based CARs confer a sensitive antigen response when targeting low affinity ligands where conventional CARs fail to respond.

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8. **Alternative viral receptors enabling SARS-CoV-2 infection**

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SARS-CoV-2 has emerged as a novel coronavirus, which has caused a global public health crisis and led to the disruption of economic, social and daily life. As is the case for many other zoonotic viruses, many aspects of its first transmission from animals to humans, as well as the spread between different species are still unknown. Therefore, there is an urgent need to characterize the underlying factors that allow viruses to successfully jump between hosts. Here, we seek to explore a novel aspect of SARS-CoV-2 transmission, by identifying previously uncharacterized alternative receptors for viral entry. These alternative receptors are not expected to generate strong infection, but rather enable the virus to jump from one species to another, followed by the adaption and subsequent onward transmission in the new host population. We will use phylogenetic, proteome and interactome analyses as well as genetic knockout and overexpression to identify these receptors in cell lines derived from humans and various animal species. Our results will provide a better insight into SARS-CoV-2 transmission and infection. Furthermore, they will help to predict which other viruses have the potential of jumping between hosts and might cause zoonotic diseases in the future.

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9. Occurrence and spread of human introduced antimicrobial resistance in a large river water system: Developing a holistic picture for the Danube River

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Background: The problem of human-induced antimicrobial resistance is an emerging concern for aquatic environments. The isolation of (facultative) pathogenic organisms with acquired antibiotic resistance, even towards last-line antibiotics, from rivers and lakes, is well documented throughout the world but large-scale and quantitative studies are often missing.

Methods: In the course of the last large international survey on the Danube River (Joint Danube Survey 4) a novel quantitative approach on the occurrence and spread of human-induced antimicrobial resistance (resistant bacteria and antibiotic resistance genes) along the whole river and its major propagation factors was applied, integrating a vast number of different environmental and microbiological parameters.

Results: Preliminary results of this comprehensive investigation showed that human faecal pollution is still the most prominent source of microbial pollution in the Danube River, potentially also introducing antibiotic resistant bacteria. The fact that faecal indicator bacteria could be quantified in substantial concentrations in river biofilms along the entire river bank indicates that due to permanent faecal pollution, human derived bacteria can also persist within the river ecosystem. Comparing an initial share of bacterial isolates (n=797) tested for acquired antimicrobial resistances a significant increase in multi-resistance as compared to previous studies was detected.

Conclusion: Based on the findings of this integrative study approach, the current understanding on the importance on the spread and stabilization of human-induced antibiotic resistance in large rivers will be highly improved. The results of this study will also be useful to guide future monitoring and management strategies.

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10. *Vibrio cholerae* (non O1/non O139) at Eastern Austrian bathing sites

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Vibrio cholerae, a potential human pathogen, is naturally occurring in aquatic ecosystems. This bacterium consists of a variety of strains and biotypes, assigned in over 200 serogroups, based on the O-antigen. Only the serogroups O1 and O139 with cholera pathogenicity factors, are able to cause severe cholera outbreaks.

However, non-toxigenic nonO1/nonO139 *Vibrio cholerae* (NTVC) strains may cause various other diseases, mainly infecting very young, elderly or immune-compromised individuals.

In the past years, several cases of NTVC infections have been documented in Austria, specifically associated with bathing activities. The increase in local infections is assumed to higher water temperatures triggered by global warming.

The aim of this study is to monitor the prevalence and abundance of NTVC at selected bathing sites in Eastern Austria along spatiotemporal environmental gradients. Solid quantitative data are obtained by culture based and culture independent (CARD-FISH/SPC and qPCR) methods, recently developed in our laboratory. Sequencing methods are applied to highlight the relationship within the microbial community, with focus on NTVC strains.

As preliminary result, we detected a geographical shift in the distribution of NTVC. Based on these data, combined with environmental information, a prediction model for the prevalence, distribution, spread and abundance of NTVC in bathing waters shall be developed.

Using this methodology, national health authorities will gain important information of the threat of VC infections in bathing waters. In addition, the applied model may also be of interest for other geographic regions.

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